Abstract

In vivo models represent important resources for investigating the physiological mechanisms underlying endocrine and metabolic disorders, and for pre-clinical translational studies that may include the assessments of new treatments. In the study of endocrine diseases, which affect multiple organs, in vivo models provide specific advantages over in vitro models, which are limited to investigation of isolated systems. In recent years, the mouse has become the popular choice for developing such in vivo mammalian models, as it has a genome that shares ~85% identity to that of man, and has many physiological systems that are similar to those in man. Moreover, methods have been developed to alter the expression of genes in the mouse, thereby generating models for human diseases, which may be due to loss- or gain-of-function mutations. The methods used to generate mutations in the mouse genome include: chemical mutagenesis; conventional, conditional and inducible knockout models; knockin models and transgenic models, and these strategies are often complementary. This review describes some of the different strategies that are utilised for generating mouse models. In addition, some mouse models that have been successfully generated by these methods for some human hereditary endocrine and metabolic disorders are reviewed. In particular, the mouse models generated for parathyroid disorders, which include: the multiple endocrine neoplasias; hyperparathyroidism-jaw tumour syndrome; disorders of the calcium-sensing receptor and forms of inherited hypoparathyroidism are discussed. The advances that have been made in our understanding of the mechanisms of these human diseases by investigations of these mouse models are described.

Introduction

In vivo models are required for investigating the physiological mechanisms underlying endocrine and metabolic disorders, and for pre-clinical translational studies and assessments of new treatments. Such in vivo models have advantages over in vitro cell culture methods, which are important for functional and molecular investigations, but are nevertheless isolated systems that are outside the context of the whole organism. Thus, in vivo models are of particular importance for the study of multi-system disorders, such as endocrine diseases, that affect multiple tissues. In recent years, the mouse has increasingly become the popular choice for developing such in vivo mammalian models, as it has many physiological systems that are similar to those in man. In addition, the coding regions of mouse and human genomes share ~85% identity (Waterston et al. 2002) and development of gene targeting methods in the mouse has facilitated the generation of mouse models for human disorders by mutagenic, transgenic and targeted approaches. Furthermore, the small size of mice, coupled with their ability to reproduce rapidly with a short generation time, yields many practical advantages for studying genetic disorders, as well as the effects of different diets and environments. Finally, the availability of inbred mouse strains helps to minimise the variations in phenotype that may occur from genetic modifiers; however, generating the same disease model in different strains enables inter-strain differences in phenotype to be studied as well as identifying the influence of genetic modifiers, which may also contribute to differential phenotypes in man. This review will focus on the main strategies that have been used for generating mouse models (Tables 1 and 2), with an emphasis on describing some of the models that are of relevance to inherited endocrine and metabolic disorders that affect parathyroid gland function, namely: multiple endocrine neoplasia (MEN) type 1 (MEN1); MEN type 2 (MEN2); MEN1-like syndrome, MEN4; hyperparathyroidism-jaw tumour syndrome (HPT-JT); disorders of the calcium-sensing receptor (CaSR) and familial forms of hypoparathyroidism (Tables 3 and 4).
Methods used for generating in vivo mouse models

Non-targeted strategies

Spontaneous mutations in mice may result in benign phenotypes such as variable coat colours, or in disorders that have similarities to diseases in man, e.g. the hyperphosphataemic rickets (Hyp) mouse, which is representative of X-linked hypophosphataemic rickets in man (Tennessee 1999). Such spontaneous mutations occur at very low frequencies, and techniques that increase the rate of mutation induction in the mouse genome by non-targeted (random) and targeted strategies have been developed (Tables 1 and 2). An early example is provided by irradiation, which generated the Gy mouse, a second model for X-linked hypophosphataemia (Tennessee 1999). Recently, chemical mutagens such as isopropyl methanesulfonate (iPMS), which was used to generate the Nuf mouse model with an activating CaSR mutation, and N-ethyl-N-nitrosourea (ENU), have been used in large-scale mutagenesis programmes. ENU, which is the most potent mutagen in mice, is an alkylating agent that primarily introduces point mutations via transfer of the ENU alkyl group to the DNA base followed by mispairing and subsequent base pair substitution during the next round of DNA replication (Acevedo-Arozena et al. 2008; Fig. 1A). Intraperitoneal injections of ENU to male mice are estimated to yield one mutation per 1–1.5 Mbp of sperm DNA (Strom et al. 1997) allowing the mutations to be inherited (Fig. 1B). ENU mutagenesis programmes utilise two complementary approaches, which are phenotype- and genotype-driven screens. In phenotype-driven screens, offspring of mutagenised mice are assessed for phenotypic variances, by a panel of morphological, biochemical or behavioural tests, in a ‘hypothesis-generating’ strategy, which may elucidate new genes, pathways and mechanisms for a disease phenotype (Acevedo-Arozena et al. 2008; Fig. 1B). By establishing appropriate matings, these phenotype-driven screens can be used to investigate for dominant or recessive phenotypes. Genotype-driven screens, in which mutations in a gene of interest are sought, are ‘hypothesis-driven’ and are feasible by available parallel archives of DNA and sperm samples from mutagenised male mice (Fig. 1B). The archived DNA samples from the mutagenised male mice are used to search for the mutations in the gene of interest, and once mutations are found in the mouse DNA, then the sperm sample for the male mouse harbouring the mutation is used for IVF to establish progeny with the mutation (Acevedo-Arozena et al. 2008). It is estimated that the probability of finding three or more mutant alleles in an archive of >5000 DNA samples is > 90% (Coghill et al. 2002), thereby enabling the gene-driven approach to be used to generate an ‘allelic series’ of mutations within one gene, which may therefore yield insights into genotype–phenotype correlations in the gene and disease of interest (Quwailid et al. 2004).

ENU mutations more frequently result in missense mutations (>80%) that may generate hypo- and hyper-morphs, although occasionally nonsense and frameshift mutations (<10%) generating knockout models may also be obtained (Barbaric et al. 2007). However, a more reliable method for generating non-targeted knockout models on a large scale is by the use of insertional mutagenesis, utilising gene traps (Collins et al. 2007a,b). Gene trap vectors usually consist of a reporter gene, either with or without a promoter, and a strong splice acceptor site, which causes any upstream exons to splice directly to the gene trap (Stanford et al. 2001; Fig. 1C). The vector is electroporated or retrovirally infected into embryonic stem (ES) cells, after which it randomly inserts into the genome. Mutagenised ES cells are then reintroduced into developing blastocysts to generate chimeraic mice, from which germline mutant mice can be bred (Fig. 2). A recent refinement of the gene trap strategy is targeted trapping, in which the vector also contains regions homologous to the targeted gene, thereby facilitating the deletion of a specific gene (Friedel et al. 2005, Collins et al. 2007b).

Table 1 Methods resulting in induction of mutations

<table>
<thead>
<tr>
<th>Method</th>
<th>Example</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>Hypophosphataemic rickets (Hyp) mouse due to mutation of Phex</td>
<td>Beck et al. (1997) and Strom et al. (1997)</td>
</tr>
<tr>
<td>Radiation</td>
<td>Gyro (Gy) mouse, which has hypophosphataemic rickets and hypercalciuria, due to deletion of Phex and spermine synthase genes on the X chromosome</td>
<td>Strom et al. (1997), Lorenz et al. (1998) and Meyer et al. (1998)</td>
</tr>
<tr>
<td>Chemical</td>
<td>Nuf mouse, which has autosomal dominant hypocalcaemia with cataracts due to an activating CaSR mutation, induced by an alkylating agent, isopropyl methanesulfonate (iPMS), which is mutagenic to testicular and epididymal sperm DNA*</td>
<td>Hough et al. (2004)</td>
</tr>
<tr>
<td>Molecular biological</td>
<td>Deletion of an allele (knockout), e.g. HPT-JT; introduction of point mutation (knockin), e.g. MEN2; transgenic that overexpresses a gene, e.g. MEN2</td>
<td>Sweetser et al. (1999), Smith-Hicks et al. (2000) and Wang et al. (2008)</td>
</tr>
</tbody>
</table>

Phex, phosphate-regulating gene with homology to endopeptidases on the X chromosome.

* N-ethyl-N-nitrosourea (ENU) is another alkylating agent that can be used.

References

Acevedo-Arozena et al. (2008); Enenhouse (1999); Hough et al. (2004); Lorenz et al. (1998); Meyer et al. (1998); Smith-Hicks et al. (2000); Strom et al. (1997).
Table 2 Some methods, with their advantages and disadvantages, for generating mouse models

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Method</th>
<th>Type of mutation</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-targeted</td>
<td>Spontaneous</td>
<td>Point mutations, small deletions, chromosomal</td>
<td>Visible phenotype, no manipulation of genome</td>
<td>Very low frequency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rearrangements</td>
<td>Easier mapping due to large rearrangements</td>
<td>Multiple genes may be affected</td>
</tr>
<tr>
<td></td>
<td>Radiation</td>
<td>Deletions, inversions, translocations, complex</td>
<td>Most potent mutants in mice; can be used for high</td>
<td>More complex mapping, may be multiple mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rearrangements</td>
<td>throughput</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemical, e.g. ENU</td>
<td>Primarily point mutations, some small deletions; hypo-</td>
<td>High targeting frequency</td>
<td>Unpredictable phenotypes</td>
</tr>
<tr>
<td></td>
<td>Molecular biological – Gene trap</td>
<td>Knockout</td>
<td>Easy to map disrupted gene; can include reporter gene</td>
<td></td>
</tr>
<tr>
<td>Targeted</td>
<td>‘Targeted trapping’</td>
<td>Knockout</td>
<td>High targeting frequency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knockout</td>
<td>Loss-of-function</td>
<td>Can include reporter gene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>Disruption of endogenous gene</td>
<td>Can mimic somatic loss-of-function; can overcome embryonic lethality of knockout</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue-specific (conditional)</td>
<td>Disruption of gene in tissue(s) of interest</td>
<td>Can mimic somatic loss-of-function; can overcome embryonic lethality of knockout</td>
<td>Requires breeding with second mouse line; some promoters have ‘leaky’ expression in other tissues</td>
</tr>
<tr>
<td></td>
<td>Inducible</td>
<td>Disruption of gene at time of choice</td>
<td>Can mimic somatic loss-of-function; can overcome embryonic lethality of knockout</td>
<td>Less than 100% knockout</td>
</tr>
<tr>
<td></td>
<td>Knockin</td>
<td>Mutation of choice including toxic gain-of-function</td>
<td>Introduction of specific mutation</td>
<td>Labour intensive; expensive</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>Activating or dominant mutations; can also inject shRNA for RNAi</td>
<td>Introduction of specific mutation or shRNA of interest</td>
<td>Random insertion into genome; transgenes often overexpressed</td>
</tr>
</tbody>
</table>

ENU, N-ethyl-N-nitrosourea; shRNA, short hairpin RNA; RNAi, RNA inhibition; ES, embryonic stem.

*All the targeted methods use molecular biological mutagenesis (Table 1). Table compiled from Stanford et al. (2001), Friedel et al. (2005), Hickman-Davis & Davis (2006), Acevedo-Arozena et al. (2008), Hacking (2008), Nguyen & Xu (2008), Fisher et al. (2009), Hall et al. (2009) and Haruyama et al. (2009).

Targeted strategies

A specific loss-of-function (i.e. knockout) of the gene of interest in the germline is generated to yield conventional targeted knockout models, as follows. A targeting construct is assembled, containing two ‘arms’ of sequence homologous to the gene of interest, which flank a positive selection cassette such as the *Escherichia coli* neomycin phosphotransferase (Neo<sup>R</sup>) gene (Fig. 3A). Integration of the Neo<sup>R</sup> gene (and therefore the targeting construct) into the ES cell genome, allows these ES cells to survive antibiotic treatment, thereby allowing selection of the ES cells that have been successfully targeted by the homologous recombination. Furthermore, replacement of an exon or exons by the Neo<sup>R</sup> cassette results in its disruption, i.e. ‘knockout’ (Fig. 3A). To further facilitate the selection of ES cells that have undergone successful targeting by homologous recombination, a negative selection cassette, such as the herpes simplex virus thymidine kinase (TK) gene, may also be used. The TK gene cassette is placed at one end of the homologous region of the targeting construct, such that if homologous recombination occurs, then the TK cassette is lost (Fig. 3A), but if non-homologous recombination occurs, then the TK cassette is retained. ES cells containing the TK cassette consistent with non-homologous recombination, will not undergo cell division when a thymidine analogue is added to the growth medium, as the thymidine analogue will be incorporated into the DNA by the TK, and thereby disrupt cell division and hence select out these ES cells. In contrast, the ES cells that have undergone homologous recombination will not have the TK cassette and will therefore not have disrupted cell division due to incorporation of the thymidine analogue, and as a result will proliferate. These correctly targeted ES cells are then used to generate chimaeric mice (Fig. 2), which are then bred with wild-type mice to yield mice who have a germline transmission of the disrupted allele, and hence are ‘knockout’ mice. Owing to the germline transmission, these mice will have one copy of the disrupted allele in all of their cells, and crossbreeding of these heterozygous knockout mice can then yield homozygous knockout mice, which will have a disruption of both alleles of the gene in all of their cells. These ‘conventional’ knockout models have proved to be very useful in studies of human diseases, although their use may be limited if the disruption of the gene in a critical organ.
### Table 3: Some in vivo mouse models for inherited endocrine syndromes

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Model type</th>
<th>Heterozygous phenotype</th>
<th>Homozygous phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN1</td>
<td>Conventional knockout</td>
<td>Tumours of pancreas, parathyroid, pituitary, gonads, adrenals, thyroid; lipomas</td>
<td>Embryonic lethal; developmental delay, craniofacial defects, haemorrhages, oedemas, neural tube defects</td>
<td>Crabtree et al. (2001), Bertolino et al. (2003a,b), Loeffler et al. (2007), Fontaniere et al. (2008), Harding et al. (2009) and Lemos et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>β-Cell/pituitary-specific knockout (Rip-Cre)</td>
<td>ND</td>
<td>Insulinomas; islet adenomas, carcinomas; small number of pituitary prolactinomas</td>
<td>Bertolino et al. (2003c), Crabtree et al. (2003a) and Biondi et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Endocrine and exocrine specific knockout (Pdx1-Cre)</td>
<td>ND</td>
<td>Glucagonomas and insulinomas in younger mice, insulinomas in older mice</td>
<td>Lu et al. (2010) and Shen et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>α-Cell-specific knockout (glucagon-Cre)</td>
<td>ND</td>
<td>Parathyroid hyperplasia</td>
<td>Libutti et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Parathyroid-specific knockout (PTH-Cre)</td>
<td>ND</td>
<td>Perinatal lethal; defects in palate, cranial bone, skeletal rib formation</td>
<td>No phenotype</td>
</tr>
<tr>
<td></td>
<td>Neural crest-specific knockout (Pax3-Cre; Wnt1-Cre)</td>
<td>ND</td>
<td></td>
<td>Scacheri et al. (2004a)</td>
</tr>
<tr>
<td></td>
<td>Liver-specific knockout (albumin-Cre)</td>
<td>ND</td>
<td></td>
<td>No phenotype</td>
</tr>
<tr>
<td></td>
<td>MEN1/MEN2-like</td>
<td>Conventional p18 knockout</td>
<td>No phenotype</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional p27 knockout</td>
<td>Organomegaly</td>
<td>Pituitary tumours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional p18/p27 double knockout</td>
<td>ND</td>
<td>Pituitary adenomas; adrenal, thyroid, parathyroid, pancreatic adenomas and carcinomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional p18/p21 double knockout</td>
<td>ND</td>
<td>Pituitary adenomas, gastric neuroendocrine neoplasias</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional Men1+/−/p18 null</td>
<td>ND</td>
<td>Earlier onset of tumours compared with conventional Men1+/−</td>
</tr>
<tr>
<td></td>
<td>MEN2A</td>
<td>Conventional Men1+/−/p27 null</td>
<td>No difference compared with conventional Men1+/−</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transgenic carrying Cys634Arg mutation in human RET cDNA</td>
<td>Thyroid C-cell hyperplasia, MTC, pancreatic cystadenomas, carcinomas</td>
<td>Renal agenesis, intestinal aganglionosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cys620Arg knockin</td>
<td>C-cell hyperplasia and adenoma, adrenal gland hyperplasia</td>
<td>Earlier onset of C-cell hyperplasia, 100% penetrance of phaeochromocytoma</td>
</tr>
<tr>
<td></td>
<td>MEN2B</td>
<td>Transgenic carrying Met918Thr mutation in human RET cDNA</td>
<td>Neuroglial tumours of sympathetic nervous system and adrenals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met918Thr knockin</td>
<td>C-cell hyperplasia; chromafin cell hyperplasia rarely progressing to phaeochromocytoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPT-JT</td>
<td>Conventional Hrpt2 knockout</td>
<td>ND</td>
<td>Embryonic lethal at E6.5; likely due to uncontrolled apoptosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tamoxifen-inducible Hrpt2 knockout (Cre-ER)</td>
<td>ND</td>
<td>Knockout during embryogenesis after E6.5; decreased growth, abnormal development of CNS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Knockout in adults: death &lt;20 days, widespread apoptosis weight loss, cachexia</td>
</tr>
</tbody>
</table>

(continued)
## Models for endocrine and metabolic disorders

To overcome such limitations, it may be useful to generate tissue-specific (i.e. conditional knockout) or time-specific (i.e. inducible knockout) models. This is achieved by refining the gene trap and ‘conventional’ knockout strategies by the addition of LoxP or flippase (FLP) recombinase target (FRT) sites in the targeting vector (Fig. 3B). LoxP and FRT sites are short DNA sequences, which are recognised and acted upon by Cre recombinase or FLP enzymes, respectively, and when inserted to flank the genomic region of interest, will result in either excision or inversion of the DNA encompassed by the LoxP or FRT sequences, depending on whether the two sequences are in the same orientation (Fig. 3B), or opposite orientations, respectively. Thus, the insertion of the LoxP and FRT sequences gives scope for several variations on the knockout mouse, including tissue- (conditional) and time-specific (inducible) knockouts (Table 2). Thus, if mice containing alleles in which the exon containing the start codon is encompassed by LoxP sites (floxed) or FRT sites (flirted), are crossed with transgenic mice expressing Cre or FLP under the control of tissue-specific promoters (e.g. the parathyroid hormone (PTH) gene promoter for parathyroid expression), the gene of interest can be knocked out in a specific tissue (Fig. 3B). Inducible models utilise a fusion protein, such as a modified ligand-binding domain of the oestrogen receptor fused to the Cre or FLP gene, which on administration of tamoxifen, translocates to the nucleus to excise the floxed allele(s), thereby allowing the gene to be knocked out at the desired time, which may be either during embryonic or neonatal development, or in adult life (Fisher et al. 2009). These conditional and inducible models have proved particularly useful in the study of tumourigenesis, to overcome the embryonic lethality that often results when tumour suppressor genes are deleted. Furthermore, the conditional and inducible strategies can be combined to generate inducible, tissue-specific models, which mimic the somatic loss of heterozygosity (LOH) of tumour suppressor genes that may trigger tumourigenesis in specific tissues (Fisher et al. 2009).

Such knockout mice have been very valuable for the study of physiological functions of proteins and the elucidation of disease mechanisms. However, knockout models are not always the most appropriate, especially if the human disease being studied is not due to a loss-of-function or null allele for the gene. Indeed, the majority of human diseases are unlikely to be due to null alleles, but are instead associated with point mutations, which may result in a constitutively active protein, dominant-negative effects or a toxic gain-of-function, as illustrated by rearranged during translocation (RET) mutations in MEN2 (see below). Thus, to generate appropriate models for these diseases, one needs to introduce the specific mutation into the mouse genome, and this may be achieved by targeted knockin or transgenic approaches (Tables 1 and 2). The generation of targeted knockin models utilises a similar approach to that described earlier for targeted knockout models, with the exception that a targeting vector

### Table 3: Continued

<table>
<thead>
<tr>
<th>Model type</th>
<th>Conventional</th>
<th>Tbx1 knockout</th>
<th>Myocardin-specific knockout</th>
<th>Foxg1-Cre; Tbx1-Cre</th>
<th>Foxg1-Cre; Foxn1-Cre</th>
<th>Foxg1-Cre; Foxn1-Cre</th>
<th>Foxg1-Cre; Tbx1-Cre</th>
<th>Foxg1-Cre; Tbx1-Cre</th>
<th>Foxg1-Cre; Tbx1-Cre</th>
<th>Foxg1-Cre; Tbx1-Cre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymic and parathyroid hypoplasia; abnormal facial structures and cleft palate; skeletal defects; cardiac OFT abnormalities</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
</tr>
<tr>
<td>Malformation of inner ear; malformation of midface; cardiac OFT abnormalities</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
</tr>
<tr>
<td>Hypocalcaemia, hypophosphataemia, enlarged parathyroids; bone abnormalities</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
</tr>
<tr>
<td>Hypocalcaemia, hypophosphataemia, enlarged parathyroids; mild bone deformities</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
</tr>
</tbody>
</table>

ND, not determined; MEN, multiple endocrine neoplasia; Rip, rat insulin promoter; Pdx1, pancreas and duodenal homeobox 1; PTH, parathyroid hormone; Foxg1, forkhead box G1; Pax2, paired box 2; Mesp1, mesoderm posterior 1; FIH, familial isolated hypoparathyroidism; GCMB, glial cells missing B.

References

Jerome & Papainamu (2003), Linsley et al. (2002, 2008), and Vitelli et al. (2001) and Arnold et al. (2006a, b).

Arnold et al. (2006a, b), Zhang et al. (2009), and Pandolfi et al. (1995), Lim et al. (2000), Miao et al. (2002, 2004), van der Wees et al. (2004), van Looij et al. (2005), and Grigorieva et al. (2010).

Miao et al. (2002, 2004), Pandolfi et al. (1995), Lim et al. (2000), van der Wees et al. (2004), van Looij et al. (2005), and Grigorieva et al. (2010).

Fisher et al. (2009).
that carries the desired mutation needs to be specifically generated (Fig. 3C). In addition, any selection cassette that is used is normally floxed and placed in an intron so that it can be excised and cause minimal effects on gene expression (Hacking 2008). The generation of transgenic models utilises a targeting construct which usually contains the cDNA carrying the mutation, together with an appropriate promoter and poly(A) sequence, which is injected into the pronucleus of fertilised mouse eggs (Haruyama et al. 2009). The transgene then undergoes random insertion into the genome, and several copies are often inserted together, which generates an overexpression model. These different strategies for generating mouse models of human diseases have greatly facilitated studies of complex inherited endocrine and metabolic syndromes that have investigated mechanisms and treatments, which would not be easily feasible in man. In addition, the development of new technologies, such as the ability to mediate deletion of genes in single cells rather than the majority of cells within a tissue, as with the Cre system, or the ability to combine several mutations within the same cells, will further advance our understanding of the mechanisms of endocrine tumourigenesis, which may involve multiple mutations.

### Multiple endocrine neoplasia type 1

MEN1 is an autosomal dominant disease characterised by the combined occurrence of parathyroid, pancreatic islet and anterior pituitary tumours (Trump et al. 1996). Some patients may also develop adrenocortical adenomas, lipomas and carcinoids (Trump et al. 1996). The MEN1 gene is located on chromosome 11q13 (Larsson et al. 1988, Friedman et al. 1989, Thakker et al. 1989, Chandrasekharappa et al. 1997, Lemmens et al. 1997), and more than 1300 MEN1 mutations, which are likely loss-of-function, have been reported (Lemos & Thakker 2008). MEN1 encodes a ubiquitously expressed 610 amino acid protein, menin, which is predominantly localised to the nucleus (Guru et al. 1998, Huang et al. 1999). Studies of menin and its interacting proteins have revealed roles in transcriptional regulation, genome stability, cell division and cell cycle control (reviewed in Thakker (2010)). The mouse Men1 gene, which is located on chromosome 19, shares 89% identity to human MEN1 (Bassett et al. 1999). This has enabled mouse models to be established, and since the majority of MEN1 mutations lead to loss-of-function of menin, studies have concentrated on knockout models (Table 3).

### Conventional Men1 knockout models

Five conventional Men1 knockout models have been generated to date, by deletion of various combinations of exons 1–8 (Crabtree et al. 2001, Scacheri et al. 2001, Bertolino et al. 2003a,b, Loffler et al. 2007, Harding et al. 2009, Lemos et al. 2009). One of these models was reported to be embryonic lethal in the heterozygous state; however, this was likely due to an aberrant transcript from the antisense strand of the phosphoglycerate kinase 1-neomycin cassette that was used to replace exons 2–4 in the model (Scacheri et al. 2001). The remaining four models show many similarities as well as some important differences. Heterozygous (Men1+/-) mice developed multiple tumours in a time-dependent manner beginning at around 9–month old, including those of the pancreas, anterior pituitary, parathyroids and adrenal tumours, as well as lipomas, which are all found in MEN1 patients. In addition, Men1+/- mice developed gonadal tumours in both male and female mice, which are not found in MEN1 patients. LOH of menin was also demonstrated in these tumours (Crabtree et al. 2001, Bertolino et al. 2003b, Loffler et al. 2007, Harding et al. 2009), consistent with the MEN1 gene being a tumour suppressor.

---

**Table 4** Mouse models for hypercalcaemic and hypocalcaemic disorders due to calcium-sensing receptor (CaSR) mutations

<table>
<thead>
<tr>
<th>Model type</th>
<th>Heterozygous phenotype</th>
<th>Homozygous phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss-of-function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional knockout (Casr−/−)</td>
<td>Hypercalcaemia, hyperphosphataemia, low plasma PTH</td>
<td>Severe hypercalcaemia, high serum PTH, parathyroid hyperplasia, bone abnormalities, neonatal death</td>
<td>Ho et al. (1995), Kovacs et al. (1998) and Garner et al. (2001)</td>
</tr>
<tr>
<td>Parathyroid-specific knockout (PT-KO)</td>
<td>Hypercalciuria, hypercalcaemia, mild hyperparathyroidism</td>
<td>Severe hyperparathyroidism, hypercalciuria, hypercalciuria, skeletal undermineralisation, neonatal death</td>
<td>Chang et al. (2008)</td>
</tr>
<tr>
<td>Osteoblast-specific knockout (COL-KO)</td>
<td>No phenotype</td>
<td>Undermineralisation of bone, multiple fractures</td>
<td>Chang et al. (2008)</td>
</tr>
<tr>
<td>Inducible chondrocyte-specific knockout (Tam-Cart-KO)</td>
<td>ND</td>
<td>Short bones</td>
<td>Chang et al. (2008)</td>
</tr>
<tr>
<td>iPMS mutant (Nuf, L723Q)</td>
<td>Hypercalciemia, hyperphosphataemia, low plasma PTH, ectopic renal calcification, cataracts</td>
<td>Hypocalcaemia, hyperphosphataemia, low plasma PTH, widespread ectopic calcification, cataracts</td>
<td>Hough et al. (2004)</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone; ND, not determined; iPMS, isopropyl methanesulfonate.
Interestingly, thyroid tumours, which have been described in MEN1 patients, but considered to be a co- incidental finding due to the high frequency of thyroid nodules in the general population (Thakker 2006), were detected in Men1<sup>−/−</sup> mice, and shown to have loss of menin expression, thereby suggesting that thyroid tumours may be part of the MEN1 syndrome (Crabtree et al. 2001, Bertolino et al. 2003b, Loffler et al. 2007, Harding et al. 2009). Only one of the models was reported to have adrenocorticotropinomas and one model was reported to have adrenal cortical tumours (Harding et al. 2009) in association with hypercortisolemia; two other models were reported to have increased circulating levels of insulin (Crabtree et al. 2001, Bertolino et al. 2003b); one model had increased serum PTH in a small proportion of mice (Bertolino et al. 2003b) and another model was reported with hypercalcemia and hypophosphatemia in the context of inappropriately normal PTH levels, consistent with primary hyperparathyroidism (Harding et al. 2009). The reasons for the differences between the various models remain to be elucidated, but may include the different background strains used. Interestingly, one of the studies assessed expression of somatostatin receptor type 2 (SSTR2) and vascular endothelial growth factor A (VEGFA), and found that expression was retained in pancreatic islet tumours and pituitary tumours, indicating that this model may be useful in testing potential new therapies such as newer somatostatin analogues and inhibitors of angiogenesis, which target SSTR2 and VEGFA respectively (Harding et al. 2009).

Homozygous knockout (Men1<sup>−/−</sup>) mice have also been studied, and are reported to be embryonically lethal between embryonic day (E) 10.5 and E14.5, with developmental delay, craniofacial defects, haemorrhages, oedema and neural tube defects (Crabtree et al. 2001, Bertolino et al. 2003a, Lemos et al. 2009), as well as a defect in the developing endocrine pancreas (Fontaniere et al. 2008). This demonstrates previously unknown roles for menin in the development of multiple tissues, which may include regulating the expression of extracellular matrix proteins that are required during organogenesis (Ji et al. 2007). Interestingly, when Men1 knockout mice were bred onto congeneric 129S6/SvEv and C57BL/6 strains, Men1<sup>−/−</sup> mice on the 129S6/SvEv background demonstrated a significantly earlier lethality than Men1<sup>−/−</sup> on the C57BL/6 background, and displayed different phenotypes, suggesting a role for genetic modifiers in menin-dependent developmental processes (Lemos et al. 2009).

**Conditional Men1 knockout models**

To overcome the problem of embryonic lethality in Men1<sup>−/−</sup> mice, several tissue-specific models have been generated (Table 3), which have the added advantage of allowing the study of tumourigenesis in individual organs. Furthermore, rather than requiring a sporadic second hit for tumourigenesis, the Men1 alleles can be deleted in all animals at the same time, thereby generating a homogenous population to study. Three pancreatic β-cell-specific Men1<sup>−/−</sup> mouse models have been generated, utilising Cre under the control of the rat insulin promoter (Rip-Cre). These mouse models demonstrated pancreatic islet cell hyperplasia, which commenced at 2 months of age, and led to the formation of insulinomas, which were associated with hypoglycaemia and hyperinsulinaemia (Bertolino et al. 2003c, Crabtree et al. 2003, Biondi et al. 2007).
In one of the models, the pancreatic islet cell adenomas progressed to carcinomas, with tumour vascularisation and down-regulation of E-cadherin and β-catenin (Bertolino et al. 2003). Tumour development in all of these models stemmed from increased cell proliferation, and interestingly, when different Rip-Cre mouse lines were used with varying expression, a higher expression of Cre was found to result in an earlier tumour formation (Crabtree et al. 2003). Other conditional models have been generated in which the Men1 allele was knocked out in both endocrine and exocrine pancreas by use of pancreatic and duodenal homeobox 1 (Pdx1)-Cre, had increased cell proliferation in endocrine but not exocrine pancreatic cells, and developed vascular insulinomas, which were associated with hypoglycaemia and hyperinsulinaemia (Shen et al. 2009). This model is of interest as it may help to elucidate the basis for tumours specifically forming in endocrine organs in MEN1.

Two pancreatic β-cell-specific Men1−/− models have also been generated, utilising a glucagon-Cre, and these have produced unexpected results. In one model, tumours expressed only glucagon, only insulin, or both glucagon and insulin in 8-month-old mice; in mice older than 12 months, only 5% of tumours expressed glucagon alone (Lu et al. 2010). However, in another model, only insulinomas developed in mice aged between 13 and 14 months (Shen et al. 2010). These differences may in part be due to transdifferentiation of α- to β-cells, or to a loss or gain of important paracrine signals which induce existing β-cells to proliferate. Further studies of these interesting models may yield insights into the less frequent occurrence of glucagonomas, compared with insulinomas, in MEN1.

A parathyroid-specific Men1−/− model has also been generated using PTH-Cre, and by the age of 9 months, >80% of mice had parathyroid hyperplasia, associated with hypercalcaemia (Libutti et al. 2003). Other tissues were normal, thereby verifying the tissue specificity of this model, and its use in elucidating the pathways of parathyroid tumourigenesis.

Other conditional models have been generated in which tissues that are not usually affected in MEN1 had the Men1 alleles deleted. These have yielded insights into the in vivo function of menin in these tissues. Thus, knockout of Men1 in neural crest cells using Pax3-Cre and Wnt1-Cre led to defects in palate, cranial bone and skeletal rib formation and perinatal lethality, thereby demonstrating roles for menin in osteogenesis and palatogenesis (Engleka et al. 2007). The generation of liver-specific null mice using albumin-Cre, which is only weakly expressed during embryogenesis (E19) and expressed fully 1–2 weeks after birth, did not lead to the development of liver tumours, although some mice developed insulinomas, which was attributed to ‘leaky’ expression of Cre in the pancreas.

Figure 2. Gene targeting by modification of embryonic stem (ES) cells. Totipotent ES cells are isolated from the inner cell mass of a blastocyst (for example from a 129Sv (shown) or C57BL/6 embryo) and cultured. The targeting vector is transferred to the ES cells, and those in which homologous recombination or integration has been successful are selected. These are injected into the inner cell mass of a blastocyst from a different mouse strain (for example C57BL/6 (shown)), which is transferred to the uterus of a pseudopregnant female. The resulting chimaeric offspring (usually males are selected) are bred with wild type, e.g. C57BL/6 mice (usually females are selected) to achieve germline transmission. m, mutant allele; +, wild-type allele.
Cyclin-dependent kinase inhibitor knockouts with MEN-like phenotypes

Cyclin-dependent kinases (CDKs) are cell cycle regulators that phosphorylate members of the retinoblastoma (RB) family and release them from binding to members of the E2F family of transcription factors (Fig. 4), which transcribe genes essential for transition to DNA synthesis (S) phase of the cell cycle (Lapenna & Giordano 2009). CDKs function in association with partner cyclins, and the associations of cyclin D with CDK4 and CDK6 are particularly important for the transition to S phase. All cell cycle components are strictly regulated, and for CDK4/CDK6, this is achieved by members of both the inhibitors of CDK4 (INK4) and CDK interacting protein/kinase inhibitory protein (Cip/Kip) families of CDK inhibitors (CDKIs), which consist of p15\textsuperscript{INK4A}, p16\textsuperscript{INK4B}, p18\textsuperscript{INK4C} and p19\textsuperscript{INK4D}; and p21\textsuperscript{CIP1/WAF1}, p27\textsuperscript{KIP1} and p57\textsuperscript{KIP2} respectively (Fig. 4). Knockout models of CDKIs have been generated and their MEN-like phenotypes have prompted \textit{in vitro} studies of the functional interactions between menin and CDKIs. Single knockouts of CDKIs have not generally yielded tumour phenotypes, which are usually found in double knockout models. For example, the p18\textsuperscript{INK4C} null and p27\textsuperscript{KIP1} heterozygous and null mice have widespread organomegaly, but only develop tumours of the anterior pituitary (Kiyokawa et al. 1996, Nakayama et al. 1996, Franklin et al. 1998). Similarly, p21\textsuperscript{CIP1/WAF1} null mice were originally reported to be free from tumours and developmental defects up to the age of 7 months (Deng et al. 1995); however, analysis of older mice showed tumour formation, although this was not in endocrine tissues (Martin-Caballero et al. 2001). However, in p18\textsuperscript{INK4C}/p27\textsuperscript{KIP1} double knockouts, pituitary adenoma development was accelerated (Franklin et al. 1998), and tumours of the adrenal, thyroid, parathyroid and testes developed, as well as pancreatic adenomas and carcinomas (Franklin et al. 2000). Furthermore, multiple tumours involving different organs were often found in the same mouse, thereby resembling MEN1 in man (Franklin et al. 2000). Similarly, p18\textsuperscript{INK4C}/p21\textsuperscript{CIP1/WAF1} double null mice have an increased prevalence of pituitary adenomas compared with p18\textsuperscript{INK4C} null, and most of the double-null mice also developed multifocal gastric neuroendocrine neoplasias, as well as lung tumours (Franklin et al. 2000). The phenotypes of these compound null mice, and their similarities to those observed in MEN1 in man has prompted several \textit{in vitro} studies exploring the functional relationships between CDKIs and menin. This has revealed that menin and mixed-lineage leukaemia (MLL) family members are recruited to the promoter regions of p18\textsuperscript{INK4C} and p27\textsuperscript{KIP1}, to activate their transcription (Milne et al. 2005; Fig. 4), which occurs via methylation of histone H3 at lysine 4 (H3K4 methylation) that is associated with the p18\textsuperscript{INK4C} and p27\textsuperscript{KIP1} promoters (Karnik et al. 2005). Both p18\textsuperscript{INK4C} and p27\textsuperscript{KIP1} are down-regulated in Men\textsuperscript{1−/−} MEFS (Milne et al. 2005), and H3K4 methylation was found to be markedly reduced or undetected in the islet tumours from Men\textsuperscript{1−/−} mice (Karnik et al. 2005).
Men1<sup>+/−</sup> phenotype, suggesting that another target of menin may interact differently with p18<sup>Ink4c</sup> and p27<sup>Kip1</sup> (Bai et al. 2007). The MEN-like phenotypes of the CDKI knockout mice have prompted a search for germline mutations in CDKIs in MEN1 patients who did not have mutations in the MEN1 gene. This revealed possible pathogenic heterozygous germline mutations, involving p15<sup>INK4A</sup>, p16<sup>INK4C</sup>, p21<sup>CIP1/WAF1</sup> and p27<sup>KIP1</sup>, with frequencies between 0.5 and 1.5%, in a small number of patients (Agarwal et al. 2009). Some of these mutations resulted in reduced expression of the CDKI in transfected HEK293 cells, whilst others resulted in decreased binding to their partner CDKs in vitro (Agarwal et al. 2009). Thus, studies of knockout mice have added novel genes and pathways to our understanding of mechanisms of endocrine tumour formation in man. Recently, some potentially oncogenic properties of p27<sup>KIP1</sup>, which are independent of its CDKI function, have also been described. These oncogenic properties appear to be related to the cytoplasmic functions of p27<sup>KIP1</sup>, which include regulation of cell motility and the actin cytoskeleton (Lee & Kim 2009). Novel mouse models will enable further exploration of these putative oncogenic properties of p27<sup>KIP1</sup>.

**Multiple endocrine neoplasia type 4**

Heterozygous germline p27<sup>KIP1</sup> mutations have been reported in four MEN1 patients without MEN1 mutations, and this variant has been termed MEN4 (Pellegrata et al. 2006, Agarwal et al. 2009). The identification of p27<sup>KIP1</sup> as an alternative MEN1 gene resulted from studies of an autosomal recessive trait in a rat model, which showed features of both MEN1 and MEN2, with phaeochromocytoma, thyroid and parathyroid hyperplasia, and pituitary adenoma, and was referred to as MENX (Fritz et al. 2002). The disease locus was mapped to rat chromosome 4 (Piotrowska et al. 2004) and subsequently found to be due to a homozygous frameshift mutation of the Cdkn1b gene encoding p27<sup>KIP1</sup>, that resulted in reduced protein levels (Pellegrata et al. 2006). A heterozygous germline nonsense mutation in Cdkn1b was subsequently identified in a patient with MEN1 who did not have a MEN1 mutation (Pellegrata et al. 2006). In vitro studies confirmed that the mutant p27<sup>KIP1</sup> protein was unstable, and failed to reach the nucleus, where it would normally interact with its target cyclin–CDK complexes (Pellegrata et al. 2006). Three other probands with MEN1 tumours but without MEN1 mutations have been reported to have loss-of-function p27<sup>KIP1</sup> mutations; however, these are likely to account for <1% of probands, and thus appear to be a rare cause of MEN4 in man (Georgitsi et al. 2007, Ozawa et al. 2007, Agarwal et al. 2009, Molatore et al. 2010).

**Multiple endocrine neoplasia type 2**

MEN2 is an autosomal dominant disease characterised by the occurrence of medullary thyroid carcinoma (MTC),...
phaeochromocytoma and parathyroid tumours. Three variants referred to as MEN2A, MEN2B and MTC-only occur. In MEN2A, MTC occurs in association with phaeochromocytoma and occasionally parathyroid tumours; in MEN2B, MTC and phaeochromocytoma occur in association with neumomas, ganglioneuromatosi of the gastrointestinal tract, medullated corneal nerve fibres and a Marfanoid habitus (Wohllk et al. 2010); in familial MTC (FMTC)—only, MTC is the sole manifestation. The gene causing the three variants, MEN2A, MEN2B and FMTC is located on chromosome 10q11.2 (Mathew et al. 1993, Norum et al. 1990, Lairmore et al. 1991), and encodes the RET proto-oncogene (Donis-Keller et al. 1987, Simpson et al. 1987, Lairmore & Saarma 2007). RET is a transmembrane receptor tyrosine kinase which has roles in organogenesis, e.g. of kidneys and neurones (Runeberg-Roos & Saarma 2007). In the presence of cofactors and ligands, that include members of the glial cell-derived neurotrophic factor family, RET dimerises to trigger autophosphorylation and intracellular signalling (Runeberg-Roos & Saarma 2007). MEN2-associated RET mutations result in constitutive kinase activity, and thus, mouse models for MEN2 have been generated by use of transgenic and knockin strategies (Table 1 and 3), as described below.

Models of MEN2A

The most common MEN2A-associated RET mutation (~52% of MEN2A cases) is the missense mutation Cys634Arg (Eng et al. 1996), and to date, three transgenic models have been generated, which carry this mutation within human RET cDNA, either in multiple tissues or in a tissue-specific manner. All three mouse models developed thyroid C-cell hyperplasia and MTC with high penetrance, and these tumours also expressed calcitonin (Michiels et al. 1997, Kawai et al. 2000, Reynolds et al. 2001). However, adrenal tumours were not found, even in a model that expressed the transgene in the adrenal gland (Michiels et al. 1997). In one of the models, the Cys634Arg-RET transgene was expressed at a high level in the pancreas, and a subset of these mice developed pancreatic cystadenomas and cystadenocarcinomas (Reynolds et al. 2001). Phosphorylated RET dimers were detected in MTCs from transgenic mice, but not in tissues without tumours such as liver and heart, even though the transgene was expressed in those tissues (Michiels et al. 1997, Kawai et al. 2000). Interestingly, in tissues without tumours, there was a high expression of the RET cofactor glycosylphosphatidylinositol-anchored co-receptor GFRα2, suggesting that this cofactor may interfere with RET dimerisation and thus tumour formation (Kawai et al. 2000). One of the MEN2A transgenic models has been crossed onto four different genetic backgrounds, and the incidence of MTCs on these different backgrounds ranged from 0 to 98% at 10 months, indicating the presence of strong genetic modifiers in the development of MTC in MEN2A (Cranston & Ponder 2003).

Another common RET mutation in MEN2A is Cys620Arg (~7% of MEN2A cases; Eng et al. 1996), and a knockin model, in which the equivalent mutation was introduced into the mouse Ret gene, has been generated as a model for MEN2A (Table 3). Heterozygous (Ret+/620R+) mice, when old, developed C-cell hyperplasia and adenomas but not MTCs, and a subset of mice developed adrenal gland hyperplasia, but not phaeochromocytomas (Yin et al. 2007). Interestingly, homozygous (Ret620R/620R) mice developed renal agenesis and intestinal aganglionosis similar to Hirschsprung disease, which is usually caused by heterozygous loss-of-function RET mutations in man (Yin et al. 2007). Further study of this model may help to elucidate the functional characteristics of RET mutations in MEN2A and their effects in both development and tumourigenesis.

Models for MEN2B

The RET mutations found in ~95% of MEN2B patients is Met918Thr (Eng et al. 1996), and transgenic and knockin models expressing this mutation have been generated (Table 3). The transgene, which was under the control of the β-hydroxylase promoter, was expressed in the developing sympathetic and enteric nervous systems, and the adrenal medulla, and the transgenic mice developed neuroglial tumours, resembling ganglioneuromas in man, in the sympathetic nervous system and adrenal glands, but not the enteric nervous system (Sweetser et al. 1999). Studies of a knockin model, which harbours the Met918Thr mutation within the mouse Ret gene, have revealed that heterozygous (Ret+/620B) and homozygous (Ret620B/620B) mice develop C-cell hyperplasia, with an earlier onset and higher penetrance in Ret620B/620B mutant mice; however, the C-cell hyperplasia did not progress to thyroid carcinoma in mice aged 10–12 months. Heterozygous Ret+/620B mice also had chromaffin cell hyperplasia, which rarely progressed to phaeochromocytoma, in contrast to the homozygous Ret620B/620B mice, which all developed phaeochromocytomas by 6 months of age, thereby demonstrating a dosage effect of the MEN2B Ret mutation which has not previously been described in man (Smith-Hicks et al. 2000). Interestingly, the homozygous Ret620B/620B mice, unlike the homozygous MEN2A mice, did not develop renal agenesis.

HPT-JT syndrome

HPT-JT syndrome is an autosomal dominant disorder characterised by the occurrence of parathyroid tumours and ossifying fibromas of the mandible and maxilla. Some patients may also develop renal abnormalities and uterine tumours (Newey et al. 2009). The HPT-JT gene, designated HRPT2, is located on chromosome 1q31.2, and demonstration of LOH in tumours has indicated that it is a likely tumour
Disorders of the CaSR

The CaSR, which is a 1078 amino acid G-protein coupled receptor with seven transmembrane domains and a large 612 amino acid extracellular domain, is predominantly expressed in the parathyroids and kidneys, and is pivotal in extracellular calcium homoeostasis by mediating alterations in the release of PTH from the parathyroids in response to changes in extracellular calcium concentrations (Thakker 2004, Egbuna & Brown 2008). CaSR mutations resulting in loss-of-function are associated with two hypercalcaemic disorders, which are familial benign hypercalcaemia, also referred to as familial hypocalciuric hypercalcaemia (FHH) and neonatal severe primary hyperparathyroidism (NSHPT; Pollak et al. 1993); whilst gain-of-function CaSR mutations result in two hypercalcaemic disorders, which are autosomal dominant hypocalcaemia with hypercalciuria (ADHH; Pollak et al. 1994, Pearce et al. 1996), and a form of the Bartter syndrome (Vargas-Poussou et al. 2002, Watanabe et al. 2002).

Models for FHH and NSHPT due to loss-of-function CaSR mutations

FHH is an autosomal dominant disorder caused by heterozygous loss-of-function CaSR mutations, and NSHPT, which is a life-threatening disorder characterised by severe neonatal hypercalcaemia, undermineralisation of bones and multiple fractures, may be caused by homozygous or de novo heterozygous loss-of-function CaSR mutations (Thakker 2004, Egbuna & Brown 2008). The loss-of-function CaSR mutations consist of nonsense mutations, frameshifting insertions and deletions and missense mutations that result in an alteration of the CaSR such that the extracellular calcium concentration at which the CaSR produces a half-maximal response (EC50) is significantly raised. To provide models for FHH and NSHPT, CaSR knockout mice were therefore generated (Ho et al. 1995; Table 4). Casr heterozygous knockout (Casr+/−) mice had modest hypercalcaemia with relative hypocalciuria and inappropriately elevated serum PTH, consistent with the features observed in FHH patients (Ho et al. 1995; Table 4). Casr−/− homozygote knockout mice had early onset of severe hypercalcaemia in association with increased serum PTH concentrations, parathyroid hyperplasia and bone demineralisation. Moreover, Casr−/− mice died between 3 and 30 days after birth (Ho et al. 1995, Garner et al. 2001). These findings in the Casr−/− mice are representative of the features found in patients with NSHPT (Egbuna & Brown 2008).

Further insights into the physiological role of the CaSR in extracellular calcium homoeostasis have been gained by breeding Casr knockout mice with Pth and glial cell missing homologue 2 (Gm2) knockout mice. Thus, Casr−/− Pth−/− mice did not have increased neonatal lethality or skeletal abnormalities (Kos et al. 2003). Furthermore, the overall mean values for serum Ca2+ concentrations and urinary Ca2+ excretion were similar in Casr−/− Pth−/− mice and wild-type control mice; however, the fine control of calcium homoeostasis was lost in the Casr−/− Pth−/− mice, which had a much larger range of serum Ca2+ concentrations. These findings demonstrate that the CaSR has PTH-independent roles in calcium homoeostasis (Kos et al. 2003). Furthermore, hypercalcaemic challenge, induced by
increased oral calcium intake, PTH infusion or dietary phosphate deficiency, resulted in a greater elevation of serum Ca\(^{2+}\) in the Casr\(^{-/-}\)Pth\(^{-/-}\) mice, which were found to have a reduced renal calcium clearance and a failure to increase serum calcitonin, compared with Casr\(^{+/+}\)Pth\(^{-/-}\) and Casr\(^{+/+}\)Pth\(^{-/-}\) mice (Kantham et al. 2009). However, infusion of PTH in Casr\(^{-/-}\)Pth\(^{-/-}\) mice further reduced the renal calcium clearance, thereby demonstrating that PTH can increase the CaSR-independent Ca\(^{2+}\) reabsorption by the kidney. Thus, it appears that PTH is necessary for the maintenance of normal serum calcium concentrations when facing a hypocalcaemic challenge, but that PTH-independent mechanisms, such as CaSR-stimulated CT secretion and increased renal Ca\(^{2+}\) excretion, are involved in maintaining normal serum calcium concentrations in response to hypercalcaemic challenges (Kantham et al. 2009).

Castr\(^{-/-}\)Gm2\(^{-/-}\) mice, which lack parathyroid glands, also do not have increased neonatal lethality, skeletal abnormalities or hypercalcemia, which are observed in Casr\(^{-/-}\)Gm2\(^{+/+}\) mice (Tu et al. 2003). Interestingly, Casr\(^{-/-}\)Gm2\(^{-/-}\) mice did not appear to demonstrate the large variability in serum Ca\(^{2+}\) levels that was observed in Casr\(^{-/-}\)Pth\(^{-/-}\) mice, and this may be due to the residual PTH production by the thymus.

The absence of bone abnormalities in Casr\(^{-/-}\)Gm2\(^{-/-}\) and Casr\(^{-/-}\)Pth\(^{-/-}\) mice, which respectively, ablate the parathyroid glands or abolish PTH, suggested that the bone abnormalities in the Casr\(^{-/-}\)Gm2\(^{+/+}\) and Casr\(^{-/-}\)Pth\(^{+/+}\) mice and in NSHPT patients are largely due to hyperparathyroidism (Tu et al. 2003). However, the expression of CaSR in bone and cartilage also suggests a role for the CaSR in the skeleton and in contributing to the bone abnormalities observed in the Casr\(^{-/-}\)Gm2\(^{+/+}\) and Casr\(^{-/-}\)Pth\(^{+/+}\) mice. An additional complication is the presence of an alternative CaSR splice variant in the Casr knockout mice, which were generated by insertion of the Neo\(^{5}\) cassette into exon 5 so as to disrupt the gene. However, this results in an incomplete knockout of the receptor because of alternate splicing of the CaSR. The alternative spliced CaSR lacks exon 5, which encodes 77 amino acid residues of the extracellular domain. This spliced receptor lacking exon 5 (Exon5(-)CaSR) is shorter, but retains the majority of the extracellular domain, all of the seven transmembrane domains and the cytoplasmic domain, and is able to compensate for the absence of full-length CaSRs in bone and cartilage (Rodriguez et al. 2005). Thus, the Exon5(-)CaSR knockout is incomplete (Rodriguez et al. 2005), and to explore further the skeletal role of the CaSR, tissue-specific knockouts of the CaSR, which lacked exon 7, that encodes the 501 amino acids, which form the seven transmembrane domains and four intracellular loops, were generated in the parathyroid, bone and cartilage (Table 4). Parathyroid-specific homozygous knockout (PT-KO) mice died ~2 weeks after birth and were much smaller than wild types. The PT-KO mice had severe marked hypercalcemia with elevated serum PTH concentrations, and increased urinary Ca\(^{2+}\) excretion, which is different to the conventional Casr\(^{-/-}\) mice, due to the presence of functional CaSR in the kidney (Chang et al. 2008). PT-KO mice also had severe undermineralisation of the skeleton, consistent with NSHPT. Heterozygous (PTHet) mice had mild hyperparathyroidism (Chang et al. 2008). Knockout of the CaSR in osteoblasts by utilising a Collagen I-Cre to generate heterozygous (COL-Het) and homozygous (COL-KO) knockout mice, revealed COL-KO mice to have a similar skeletal phenotype to the PT-KO mice, with multiple fractures and profound undermineralisation, due to a lack of differentiation and increased apoptosis, of osteoblasts (Chang et al. 2008). Homozygous knockout of the CaSR in growth plate cells caused embryonic lethality at ~E12–E13, and to overcome this, an inducible growth plate-specific knockout was generated via the use of an ER-Cre. The CaSR was knocked out in growth plates before birth, and this again resulted in bone defects such as short bones, as well as a decrease in mature and terminally differentiated chondrocytes (Chang et al. 2008). Thus, these tissue-specific knockout models demonstrate a role for the CaSR in bone and calcium homoeostasis, and indicate that the skeletal abnormalities seen in patients with NSHPT may not be solely due to hyperparathyroidism.

Models for ADHH due to gain-of-function CaSR mutations

Gain-of-function CaSR mutations are associated with the disorder of ADHH. The hypocalcaemia is usually mild and asymptomatic, but may sometimes be associated with tetany and seizures. Hyperphosphataemia and hypomagnesaemia are also notable features, and the circulating PTH concentrations are usually in the low-normal range (Pearce et al. 1996). The CaSR mutations resulting in ADHH are invariably heterozygous missense mutations that result in a lower EC\(_{50}\). To generate a mouse model for ADHH and such an activating CaSR mutation, two possible strategies could be utilised, which are a knockin approach or searching for a chemically induced mutation (Table 2 and Figs 1 and 3). Indeed, a search of mice derived from use of the chemical iPMS to produce mutants, identified a mouse model for ADHH (Table 4). This mouse model, which was first noted to have opaque flecks of the lens and designated nuclear flecks (Nuf; Hough et al. 2004), has hypocalcaemia and hyperphosphataemia in association with inappropriately low plasma PTH concentrations. In addition, mutant mice may also have widespread ectopic calcification and suffer from sudden death (Hough et al. 2004). These phenotypic features in the mutant Nuf mice are due to a CaSR missense mutation, Leu723Gln, which results in a reduced EC\(_{50}\), consistent with an activating CaSR mutation (Hough et al. 2004). Thus, the Nuf mouse is a model for ADHH due to an activating mutation of the CaSR, and this model may be an important tool to understand the mechanisms of ectopic calcification.
Inherited hypoparathyroidism

Hypoparathyroidism, which causes hypocalcaemia and hyperphosphataemia, and is associated with tetany and seizures, can occur as an idiopathic endocrinopathy or as part of a syndrome such as the DiGeorge syndrome (DGS), or the hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome. Forms of familial isolated hypoparathyroidism (FIH) with autosomal dominant, autosomal recessive and X-linked recessive modes of inheritance have been described.

Model for the DGS due to T-box 1 mutations

DGS is characterised by the occurrence of hypoparathyroidism, immunodeficiency, congenital heart defects and deformities of the ear, nose and mouth. The disorder arises from a congenital failure in the development of the derivatives of the third and fourth pharyngeal pouches, with resulting absence or hypoplasia of the parathyroids and thymus. DGS was reported to be associated with unbalanced translocations and deletions involving chromosome 22q11.2, and further studies identified mutations of the transcription factor, T-box 1 (TBX1), in patients with DGS (Gong et al. 1996, Yagi et al. 2003). Mice that are deleted for Tbx1 have developmental abnormalities of the pharyngeal arches (Table 3). Thus, Tbx1+/− mice have cardiac outflow tract abnormalities, which are defects of the fourth branchial pouch (Jerome & Papaioannou 2001, Lindsay et al. 2001, Vitelli et al. 2002). However, Tbx1−/− mice have all the developmental anomalies of DGS due to third and fourth branchial defects which include: thymic and parathyroid hypoplasia; abnormal facial structures and cleft palate; skeletal defects and cardiac outflow tract abnormalities (Jerome & Papaioannou 2001, Lindsay et al. 2001, Vitelli et al. 2002). The basis of the phenotypic differences between DGS patients, who are heterozygous and the Tbx1+/− mice remain to be elucidated. It is plausible that TBX1 dosage, together with downstream genes that are regulated by TBX1 could provide an explanation, but the roles of these putative genes in DGS remain to be elucidated. Several tissue-specific knockout models of Tbx1 have also been generated (Table 3). Knockout of Tbx1 in the pharyngeal endoderm led to neonatal death with malformations identical to Tbx1−/− mice, and likely due to failure of pharyngeal pouch outgrowth (Arnold et al. 2006b). Inactivation of Tbx1 in the otic vesicle led to an absence of the inner ear (Arnold et al. 2006a). Mesoderm-specific knockout of Tbx1 led to multiple phenotypes that included malformation of the inner ear, defective pharyngeal patterning, cardiovascular defects, defective development of the proximal mandible and thyroid hypoplasia (Zhang et al. 2006, Braunstein et al. 2009, Lania et al. 2009, Aggarwal et al. 2010). An ‘allelic series’ of Tbx1 expression showed tissue-specific dosage effects, demonstrating that cardiac outflow tract development is more susceptible to loss of Tbx1 than craniofacial development (Hu et al. 2004). These models have helped to elucidate the roles of TBX1 in organogenesis and DGS, and some of the transcriptional cascades required for development of these structures.

Model for the HDR syndrome due to GATA3 haploinsufficiency

HDR is an autosomal dominant disorder, characterised by hypocalcaemia with undetectable or inappropriately normal serum concentrations of PTH; bilateral sensorineural deafness and variable renal abnormalities, including cysts, hypoplasia and aplasia (Bilous et al. 1992, Muroya et al. 2001, Nesbit et al. 2004, Ali et al. 2007). HDR is due to GATA3 haploinsufficiency (Van Esch et al. 2000), and studies of Gata3 knockout mice have revealed that these develop features of HDR (Table 3). Heterozygous (Gata3+/−) mice initially appeared phenotypically normal (Pandolﬁ et al. 1995); however, further studies have shown that Gata3+/− mice develop two of the three components of the HDR syndrome. When challenged with a low-calcium diet, Gata3+/− mice were unable to mount the appropriate parathyroid response, and thus had a lower plasma PTH and calcium than their wild-type littermates, as well as smaller parathyroid glands with lower proliferation rates (Grigorieva et al. 2010). Gata3+/− mice had smaller parathyroid–thymus primordia at E11.5, with fewer cells expressing Gcm2, and Gata3−/− mice had no primordia after E12.5, with a complete absence of Gcm2-expressing cells, indicating a defect of parathyroid gland development in both Gata3+/− and Gata3−/− mice (Grigorieva et al. 2010). Indeed, GATA3 bound to a functional double GATA motif in the GCMB promoter in human parathyroid adenoma cells, demonstrating a direct link between GATA3 and GCMB as part of a critical transcriptional cascade in parathyroid development (Grigorieva et al. 2010). Gata3+/− mice had hearing loss starting in the early postnatal period, and progressing through adulthood (van der Wees et al. 2004). This hearing loss was found to be due to cochlear abnormalities starting with outer hair cell dysfunction at the apical cochlea, and progressing to the base of the cochlea (van der Wees et al. 2004, van Looij et al. 2005). Gata3 null (Gata3−/−) mice are embryonically lethal and died at E11–E12 with internal bleeding, growth retardation, severe deformities of the brain and spinal cord, defective haematopoiesis, lack of T-cell differentiation and a lack of noradrenaline in the sympathetic nervous system (Pandolﬁ et al. 1995, Lim et al. 2000). The lack of noradrenaline, due to an absence of tyrosine hydroxylase and dopamine β-hydroxylase enzymes, was overcome by feeding catecholamine intermediates to pregnant dams, thereby partially rescuing the Gata3−/− embryos to E12.5–E16.5 (Lim et al. 2000). This revealed other phenotypic abnormalities, including thymic hypoplasia, renal hypoplasia and defects in structures derived from cephalic neural crest cells (Lim et al. 2000, Grote et al. 2006). Rescue of Gata3−/− embryos to E18.5 by administration of adrenergic receptor agonists also revealed cardiac defects including ventricular and aortic arch defects.
(Raid et al. 2009). These studies reveal the role of GATA3 and catecholamines in organogenesis and in the HDR syndrome.

**Models for FIH due to PTH and GCMB mutations**

Both autosomal dominant and autosomal recessive forms of FIH have been found to be due to mutations in PTH and GCMB, which is a member of a small family of GCM transcription factors whose expression appears to be restricted to the developing and adult parathyroid glands (Kim et al. 1998). FIH–associated PTH and GCMB mutations likely lead to a loss–of–function, and knockout mice for Pth and the mouse GCMB homologue Gcm2, develop hypoparathyroidism (Table 3). Pth+/− mice were viable with no apparent phenotypic abnormalities. However, PTH null (Pth−/−) mice had enlarged parathyroid glands with an absence of PTH expression, but with substantial CaSR expression. Pth−/− mice also had abnormal skull formation with enhanced mineralisation, along with shortening of the long bones and other bone abnormalities (Miao et al. 2002). When maintained on a normal calcium diet, Pth−/− mice developed hypocalcaemia and hyperphosphataemia, consistent with hypoparathyroidism, that was associated with an increased serum 1,25(OH)2D3 concentration. Moreover, when given a low–calcium diet, serum 1,25(OH)2D3 concentrations further increased in the Pth−/− mice, and this led to increased bone resorption and a maintenance of serum calcium at the expense of osteopenia (Miao et al. 2004).

Mice lacking a copy of Gem2 (Gem2+/−) appear normal, viable and fertile. However, Gem2−/− mice have an absence of parathyroid glands, with hypocalcaemia and hyperphosphataemia, consistent with hypoparathyroidism. However, in contrast to Pth−/− mice, PTH was detected in the serum and this PTH was produced by a cluster of cells in the thymus (Gunther et al. 2000). A detailed analysis of Gem2−/− embryos showed that a parathyroid domain was initially formed, with transient expression of CaSR, before being lost by E12.5 due to apoptotic cell death. GCM2 and GATA3 form part of a transcriptional cascade that facilitates parathyroid development (Liu et al. 2007, Grigorieva et al. 2010).

**Conclusions**

Mouse models for inherited endocrine and metabolic syndromes have been generated by a variety of strategies, and have provided new and valuable insights into the pathogenesis of these complex disorders. These studies have been used both to generate models for disorders with known genetic causes, and to identify novel genes causing mouse phenotypes similar to those in man, thus increasing our understanding of the molecular and genetic mechanisms and pathways in these disorders. Furthermore, the resources have been developed to facilitate the generation of mouse models for human diseases (Table 5), and this will help to increase our understanding of the molecular and systemic mechanisms underlying human disorders, as well as providing pre-clinical models for assessing therapies.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

S E P and R V T are grateful to the Medical Research Council UK (grants G9825269 and G1000467) for support.

### Table 5 Some online resources and databases that provide details for generating mouse models

<table>
<thead>
<tr>
<th>Resource</th>
<th>Contents</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Genome Informatics (MGI), The Jackson Laboratory</td>
<td>Data from genome, expression, tumour biology and ontology databases, disease models searchable by gene, phenotype and system. Includes International Mouse Mutagenesis Consortium (IMMC), International Complex Traits Consortium (CTC) and International Gene Trap Consortium (IGCT)</td>
<td><a href="http://www.informatics.jax.org/">http://www.informatics.jax.org/</a></td>
</tr>
<tr>
<td>International Mouse Strain Resource (IMSR), The Jackson Laboratory</td>
<td>Database of mouse strains and stocks, including inbred, mutant and genetically modified mice</td>
<td><a href="http://www.findmice.org/">http://www.findmice.org/</a></td>
</tr>
<tr>
<td>International Knockout Mouse Consortium</td>
<td>Database of available knockout ES cell lines; includes Knockout Mouse Project (KOMP), European Conditional Mouse Mutagenesis Programme (EU/COMM), North American Conditional Mouse Mutagenesis Project (NorCOMM) and Texas A&amp;M Institute for Genomic Medicine (TIGM)</td>
<td><a href="http://www.knockoutmouse.org/or">http://www.knockoutmouse.org/or</a> <a href="https://www.komp.org/ikmc/">https://www.komp.org/ikmc/</a></td>
</tr>
<tr>
<td>Mouse Phenome Database, The Jackson Laboratory</td>
<td>Baseline phenotypic data from different inbred mouse strains</td>
<td><a href="http://phenome.jax.org/">http://phenome.jax.org/</a></td>
</tr>
</tbody>
</table>
References

genom.9.081307.164224)


Models for endocrine and metabolic disorders  ·  S E PRET and R V THAKER 227


Hickman-Davis JM & Davis IC 2006 Transgenic mice. Paediatric Respiratory Reviews 7 49–53.


www.endocrinology-journals.org


Received in final form 24 June 2011
Accepted 15 July 2011
Made available online as an Accepted Preprint 15 July 2011