16 Animal Models

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Introduction

The use of animal models has been an essential aspect of nearly all areas of nephrological research since its earliest days. Research on kidney formation and malformation, physiology and pathophysiology, immunological injury, and tolerance or transplant rejection all depend on the use of animal experimentation. This chapter will emphasize genetic approaches that utilize animals, as this area has shown the great progress in the development of novel technologies, that have had great impact in all areas of nephrology.

Institutional Oversight

There is increasing public awareness of the use of animals in research, and with this comes increasing concern about the appropriateness of the use of animals, and whether much of the research that does involve animal models could be accomplished using non-animal models. Therefore it is important to note that all animal research in the United States and presumably in most other countries must be evaluated by institutional committees before any experimentation may commence. Furthermore, the United States Department of Agriculture (USDA) provides constant oversight through the use of frequent and usually unannounced visits to animal facilities with research institutions. These regulatory committees and agencies are charged with evaluating animal protocols to make certain that animals are used in an ethical manner, with proper use of anesthetics or analgesics to minimize or eliminate any source of pain during experimentation. They are also charged with verifying that animals are indeed required for the specific research in question, that large animals are not used when smaller ones would suffice, and that the investigators are trained and knowledgeable about proper use of animals. Despite these several layers of oversight, in the end it is up to the principal investigator to be thoughtful about whether their intended experimental approach will yield sufficiently important and worthwhile results to justify the use of laboratory animals.

Animal Models of Kidney Disease

The selection of an animal model for some aspect or type of kidney disease takes several factors into consideration. Most importantly, the similarity to human disease that can be observed in a particular model is taken into account. Other important factors include the cost of the animals involved: the cost of maintaining animals larger than rodents increases dramatically with size, and the numbers of animals that can be studied consequently decreases. For this reason, some studies may begin with a rodent model, and then progress to a larger model once the rodent model establishes the feasibility of the hypothesis under study. The size of an animal may be important to the extent that it affects the ability to perform surgical manipulations or physiological measurements. However, since it has become increasingly desirable to obtain physiological measurements on various strains of knockout mice, the equipment available to perform these measurements has improved and become commercially available.

Genetic Models

Animal models of disease that have a genetic basis may either result from spontaneous or induced mutations. Spontaneous mutations or phenotypes are those noticed either by chance or through the directed observation of large numbers of mice, that were not otherwise treated to induce a mutation. In contrast, induced mutations are those resulting from the treatment of mice with irradiation or mutagenic agents known to introduce point mutations or deletions into the genome.

The past 20 years have witnessed an explosion in the use of genetic approaches to understand development and physiology, and thus they will receive appropriate emphasis in this chapter. Several genetic approaches are available for use with animal model systems. A gene of interest may be mutated using gene targeting, or expressed in transgenic mice in such a way to interfere with its normal function. On the other hand, it is possible to start with a phenotype of interest, which could either be obtained as a spontaneous mutation or from mice treated with

a mutagen, and an effort is made to identify the mutated gene responsible for the phenotype. Genetic approaches using gene targeted or transgenic mice are useful for a wide variety of developmental and physiological studies in which there is a need to study the function of a known gene.

Genetic Approaches with known Genes-Genotype to Phenotype

Gene Targeting

Gene targeting was originally used to introduce a deletion or interruption into a gene of interest, using the scheme shown in *Fig. 16-1*, such that it could be determined whether mice would be able to develop in the absence of that gene's function. In cases where a gene was shown not to be essential for development, the homozygous mutant mouse might serve as a useful model in which to study the role of a specific gene in a physiological or disease process. For example, targeted deletions of the Wt1 (1), Pax2 (2), GDNF (3–5), Wnt4 (6), and BMP7 (7, 8), among others, showed these genes to be essential for various aspects of early kidney development. On the other hand, the absence of many immunology-related genes does not result in any developmental impairment, but these mice have served as useful models to study the role of the immune system in transplant rejection.

The advent of gene targeting was made possible through the use of two technologies developed mainly in the 1980s. The first was the development of tissue culture conditions that allowed embryonic stem (ES) cell lines to be grown indefinitely in culture while retaining their totipotency (9). ES cells grown in culture could then be introduced into mouse preimplantation embryos or blastocyts, and become fully integrated into those embryos such that their descendant cells would give rise to all developmental lineages that are found in adult mice (10). The second technology involved the use of homologous recombination to introduce mutations into mammalian genes (11–13). As shown in *Fig. 16-1*, when long stretches of genomic DNA in recombinant DNA constructs are introduced into cells in culture, this DNA will, at variable and often quite low frequency, recombine into the locus from which the genomic DNA was originally derived. Therefore homologous recombination of the correctly designed genomic fragment can be used to introduce a deletion or insertion into a genomic locus, that renders the gene unable to be expressed. This ES cell would in essence be heterozygous for a mutation in the targeted gene, and heterozygous ES cells can be isolated and expanded to provide a population for injection into blastocysts. Therefore, by combining the ES cell technology and homologous recombination, it became possible to target mutations into genes in ES cells, and then introduce ES cells carrying these mutations into blastocysts, finally obtaining a mutant adult mouse.

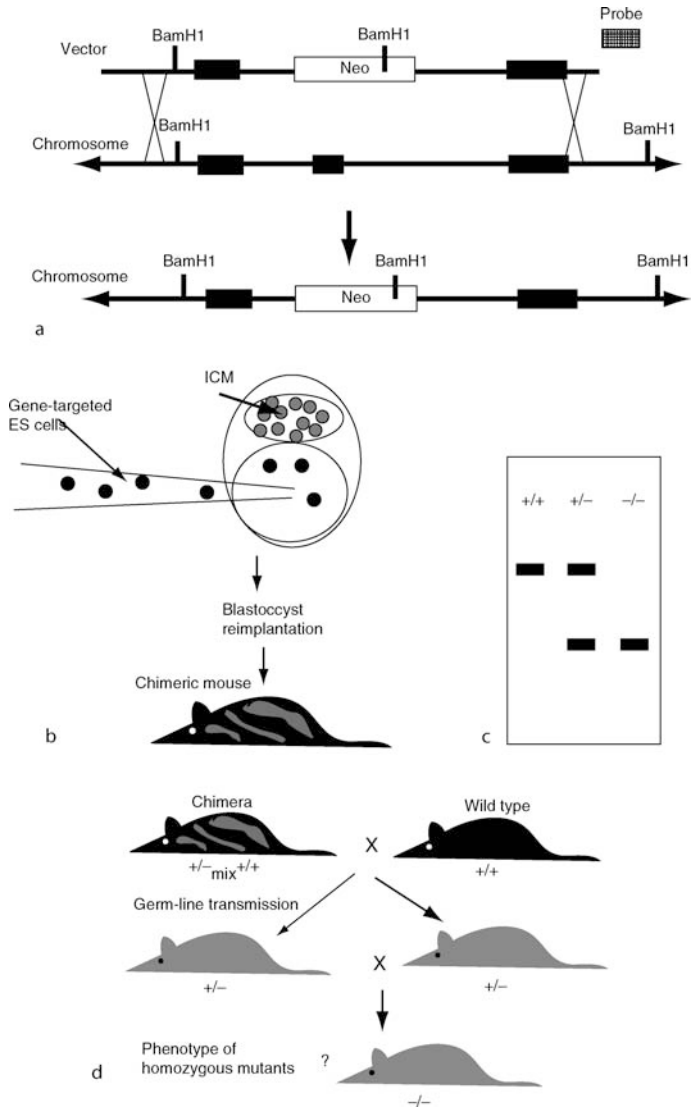
In a typical experiment, gene targeted ES cells would contain one mutated allele and one normal or wild type allele for the gene under study. The targeted ES cells would be injected into preimplantation blastocysts, and groups of these blastocysts would be introduced into female mice that were previously hormonally primed to allow implantation of the injected blastocysts into their uteri, to begin a pregnancy. The resultant mice from these injections are termed "chimeras," because any specific cell is either derived from an ES cells or the original injected embryo, i.e., the chimeric mouse essentially has four parents, the male and female that provided the blastocyst, and the male and female that provided the embryo from which the ES cell line in use was originally derived. In the best cases, a chimera might be nearly entirely derived from the ES cells. Among the tissues that ES cells contribute to are the germ cells: spermatocytes or oocytes. When ES cells heterozygous for a mutation are used to make a chimera, germ cells derived from the ES cells have a 50% chance of carrying the mutant rather than the wild type allele. Therefore, mating a chimeric and wild type mice can result in some of the offspring being true heterozygotes for the mutated gene. After obtaining both male and female heterozygotes, they can be mated to obtain homozygous mutant embryos or mice, depending on whether or not the gene is essential for development.

Conditional Gene Targeting

The process described in the preceding section results in the inactivation of a target gene from the beginning of embryogenesis. In this situation, an embryo will become non-viable at the first point at which expression of the inactivated gene becomes essential for survival. However, it may be highly desirable to study the function of a gene product in many later events during development or adult life. Conditional gene targeting allows the inactivation of a gene in particular tissues or at particular times during development or adult life (14–16). This technology has been developed more recently, and has proved more difficult to employ on a widespread basis thus far, for reasons that will be discussed.

Figure 16-1

Gene targeting in mice. (a) The scheme for targeting a deletion of an exon in embryonic stem cells. Exons are shown as black boxes along a chromosome. Restriction sites for restriction enzyme BamH1A are shown. The replacement vector is constructed such that the neomycin resistance gene (neo) is shown as an open box, in place of one of the exons. An external probe specifically does not overlap with the replacement vector. A double homologous recombination results in then integration of the vector into the chromosome, thus replacing the exon with the Neo gene. The BamH1 site within the neo gene results in a shorter BamH1 restriction fragment detected by probe after homologous recombination. (b) ES cells can be injected through a micropipette into a blastocytst, where they become part of the inner cell mass. The injected blastocyst is introduced into the uterus of a hormonally primed mouse, and gives rise to a chimeric mouse, partially derived from the ES cells, and partially from the original inner cell mass cells. If the ES cells and blastocysts are derived from strains with different coat colors, then the chimeric mouse will have a variegated coat color pattern on its fur, providing an indication of its overall extent of chimerism. In the best cases, the resultant mouse is nearly entirely derived from ES cells. (c) shows a possible pattern obtained in a Southern blot, based on the scheme shown in (a), using the external probe. A wild type mouse shows only the longer band. A heterozygous mouse shows both the wild type and gene targeted band, and the homozygous mutant shows only the shorter band, due to the presence of the BamH1 site in the neo gene. (d) the mating involved in obtaining germ line transmission of the mutation and subsequently obtaining homozygous mutant mice.



The general approach to conditional gene targeting is shown in *Fig. 16-2*. This is a variation on traditional gene targeting, in that it also relies on homologous recombination to introduce a segment of recombinant DNA into the locus of a gene in ES cells. However, whereas traditional gene targeting inactivates the gene, conditional gene targeting must modify the gene such that it can be expressed until such time as its inactivation is desired. The most commonly used approach involved the insertion of LOX sites, which are 34 base pair sites involved in site-specific recombination by Cre recombinase, and enzyme originally derived from a bacteriophage (15). Since LOX sites are rather small, it is usually possible to insert them in introns where they have no effect on gene expression. By placing two LOX sites in a gene to flank an exon, Cre can be used to inactivate a gene by recombining out the DNA segment containing the exon that was situated between the two LOX sites, thus inactivating the gene. There are experimental approaches for expressing Cre in temporally and spatially specific manners or both. Spatial or lineage specific expression of Cre is most often obtained by placing the Cre cDNA downstream of a known tissue specific promoter. Sometimes this is achieved by using homologous recombination to insert the Cre gene into the genomic locus of a gene with known tissue specific expression, such that Cre replaces the first exon of that gene. Temporally specific expression of Cre has proved more difficult to obtain. One approach is to regulate Cre using the tetracycline system for inducible gene expression (17). The other approach makes use of a fusion protein consisting of Cre and a portion of the estrogen receptor that confers steroid mediated nuclear localization (18, 19). The latter is modified to bind tamoxifen or tamoxifen-derivatives instead of estrogen. The Cre-modified estrogen receptor fusion protein will remain in the cytoplasm and therefore not be able to mediate site-specific recombination of LOX sites, until tamoxifen is administered to the mouse to induce nuclear translocation of the Cre fusion protein. This system can be used to induce recombination in embryos, when tamoxifen is administered to pregnant mice. The major obstacle to employing conditional gene targeting on a widespread basis is the availability of promoter/enhancer elements that are able to confer robust tissue or cell-lineage-specific expression of Cre recombinase. However, an increasing number of mouse strains are available that express Cre recombinase in various cell lineages within the developing and adult kidney (20). It is possible to obtain conditional knockouts restricted to nephron progenitor cells (21), podocytes (21, 22–24), proximal tubules (25–27), thick ascending limb (28), ureteric bud (29), juxtaglomerular cells, and collecting ducts (30). It is also possible to obtain vascular knockouts (31–33), though not restricted to the kidney.

As more tissue-promoter elements become available, conditional gene targeting promises to have a large impact on genetic approaches to kidney disease. As noted above, there are many genes expressed both in developing and adult kidney, where the knockout of the gene results in embryonic lethality. This precludes study of how that product of that gene might function in postnatal kidneys, or why a mutation in that gene leads to kidney disease in humans. It also raises the question of why humans carrying such mutations are able survive, albeit with a genetic disease, when mice carrying mutations in the same gene do not survive embryogenesis. Sometimes this is simply because mice and humans differ in their respective requirements for specific genes, but more often, it is because humans with genetic disease often have point mutations that lead to partial loss of function, whereas mouse knockouts often involve complete loss of function mutations. Conditional gene targeting can sometimes offer a solution to this problem, by allowing normal gene expression during embryogenesis, and then inactivating a gene in adult mice. Alternatively, there are variations on the Cre-LOX approach that allow the introduction of point mutations into mice. The introduction of point mutations into mice has been greatly facilitated by recent advancements that facilitate homologous recombination into BACs (Bacterial Artificial Chromosomes) in E. Coli (*Fig. 16-3*) (34–36). BACs are used as they contain large amounts of genomic DNA, and thus are ideal for use as gene targeting vectors. The longer length of BACs compared with shorter genomic clones should improve the frequency of homologous recombination in ES cells.

Animal Models using RNAi Approaches

RNAi technologies have had great impact across the breadth of molecular biology and the study of disease over the past 10 years (37). RNAi is based on a mechanism common to both plant and animals whereby a short strand of RNA, that is associated with a set of proteins known as the RISC complex, hybridizes to a complementary sequence in a mRNA, causes degradation or inhibition of translation of the mRNA (38, 39). In the natural setting, these small RNAs are encoded in the genome as microRNAs. The same molecular machinery used by microRNAs to inhibit expression of mRNAs can be

Figure 16-2

Conditional gene targeting. The targeting vector is different from the previous figure in that LOX sites flank the exon that will eventually be deleted, and the Neo gene is flanked by Frt sites. The vector is incorporated into the chromosome through homologous recombination, and ES cells with this knock-in are used to make chimeric mice, and germline transmission is obtained. Although the LOX sites should not interfere with expression of the gene, the Neo gene is likely to interfere with normal gene expression. However, in most cases mice will tolerate one inactive gene, as long as the other allele is functional. After obtaining heterozygous mice, they are mated with Flp – deleter mice, that express Flp recombinase in germ cells. Flp will recombine the FRT sites and eliminate the neo gene. Mice without the neo gene, but still containing the exon flanked by LOX sites, are mated with mice expressing Cre in a particular tissue or cell type, or expressing an inducible Cre, to obtain the conditional knockout. The breeding scheme shown in the figure is oversimplified. In the actual experiment, a more complicated breeding scheme is required to obtain a mouse that is homozygous for alleles with LOX sites, and that also has the Cre-expressing transgene. An alternative is to breed mice with the conditional allele with mice carrying a traditional knockout. This has the advantage that to obtain the conditional knockout, Cre must only recombine one, and not two, pairs of LOX sites in each cell.

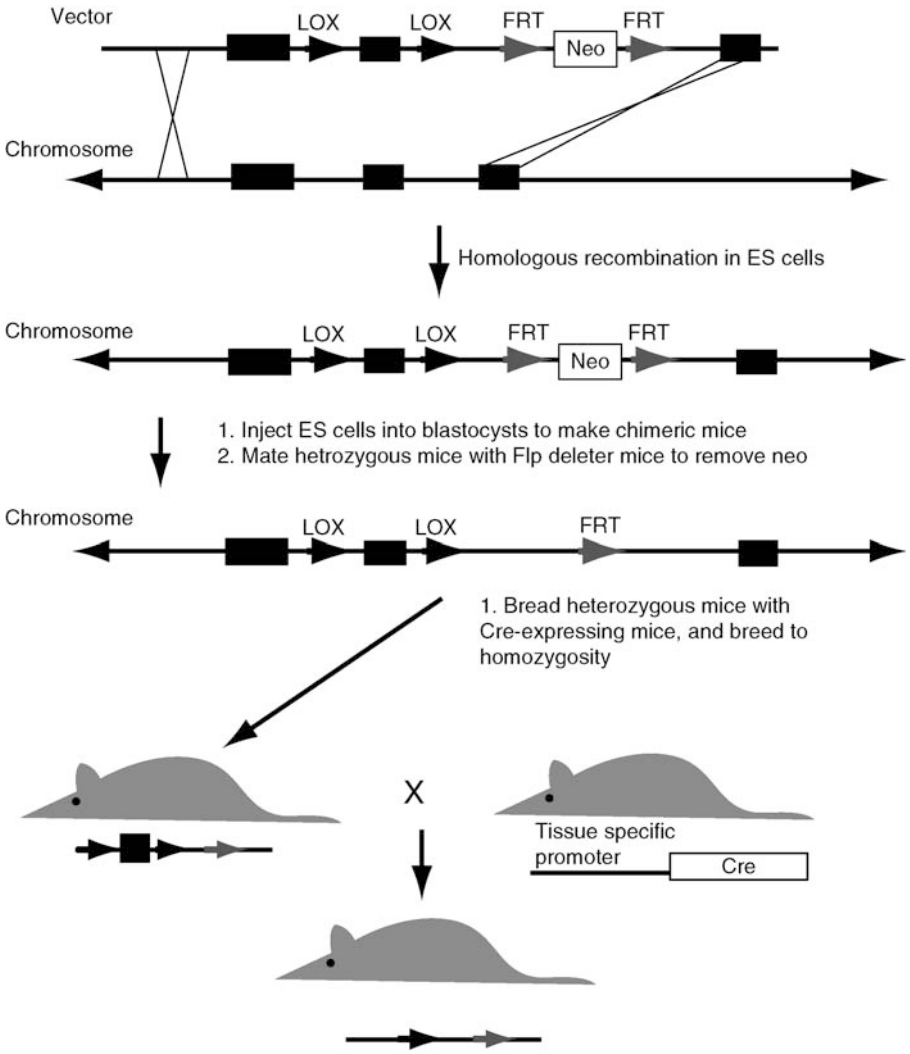
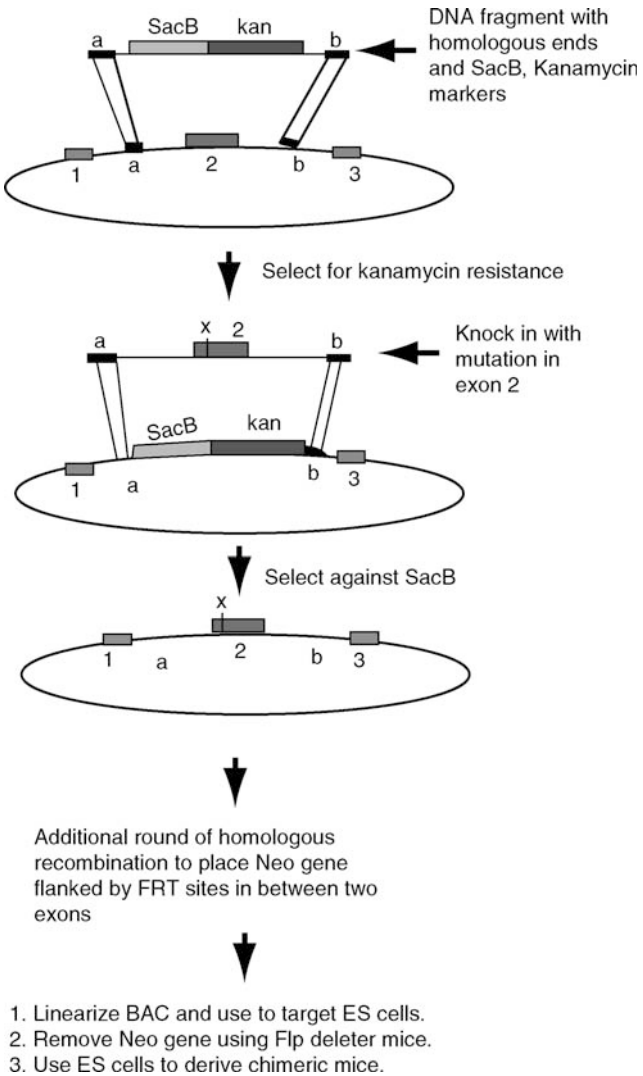


Figure 16-3

Gene targeting using BAC clones. Homologous recombination is done in E. coli instead of in ES cells. In the first step, a DNA fragment is prepared that contains the kanamycin resistance positive selectable marker and the SacB negative selectable marker, and which also contains homologous ends (A and B, each about 50–60 bp) is introduced into E. coli. This fragment can usually be prepared by PCR, using primers that contain the homologies to the genomic region, and also to a vector containing the selectable markers. Usually, a strain of E. coli is used that allows transient activation of the enzymes required for homologous recombination. Selection for kanamycin resistance will obtain BAC clones where the selectable markers have recombined into the BAC. In a second round of homologous recombination, A DNA fragment with the same homologous ends but containing a mutated exon 2, denoted by the "X," is introduced into the E. coli containing the BAC. Selection against SacB will obtain BACs in which the mutated exon 2 has replaced the selectable markers. A third round of homologous recombination is used to insert the neo gene, flanked by FRT sites, so that the BAC can be used for homologous recombination in ES cells. As shown, this scheme is used to introduce point mutations or small deletions into a gene. It can also be used to construct conditional knockout vectors, similar to those shown in *Fig. 16-2*. An additional use is to knock-in a Green Fluorescent Protein (GFP) or β-galactosidase (LacZ) reporter gene into a locus to obtain information about patterns of gene expression.



co-opted for use by siRNA (silencing RNA) or shRNA (short hairpin RNA) that are ectopically expressed in cells. siRNA refers to RNA duplexes that are usually obtained commercially and transfected into cells. shRNA refers to small RNAs that are expressed from plasmids or viral vectors that are introduced into cells. A major addition to the arsenal of approaches involving transgenic mice involves the derivation of transgenic mice that express shRNA molecules capable of reducing levels of mRNA for specific genes in specific tissues or cell lineages (38–41). shRNAs can be expressed either constitutively, or conditionally using the Cre-Lox system (*Fig. 16-4*). An advantage of shRNA technology over gene knockouts is that it is quicker and cheaper, as mice do not need to be bred to homozygosity and also to carry the Cre transgene. Instead, a resulting phenotype can be obtained by mating a Cre-expressing mouse with a mouse carrying a conditional shRNA transgene, and examining the first generation offspring carrying both transgenes. The major disadvantage in comparison with gene knockouts is that expression of the target gene may be variably reduced, and it may be necessary to examine several shRNA transgenic lines in order to obtain ones that demonstrate efficient knockdown of the desired gene.

Transgenic Mice

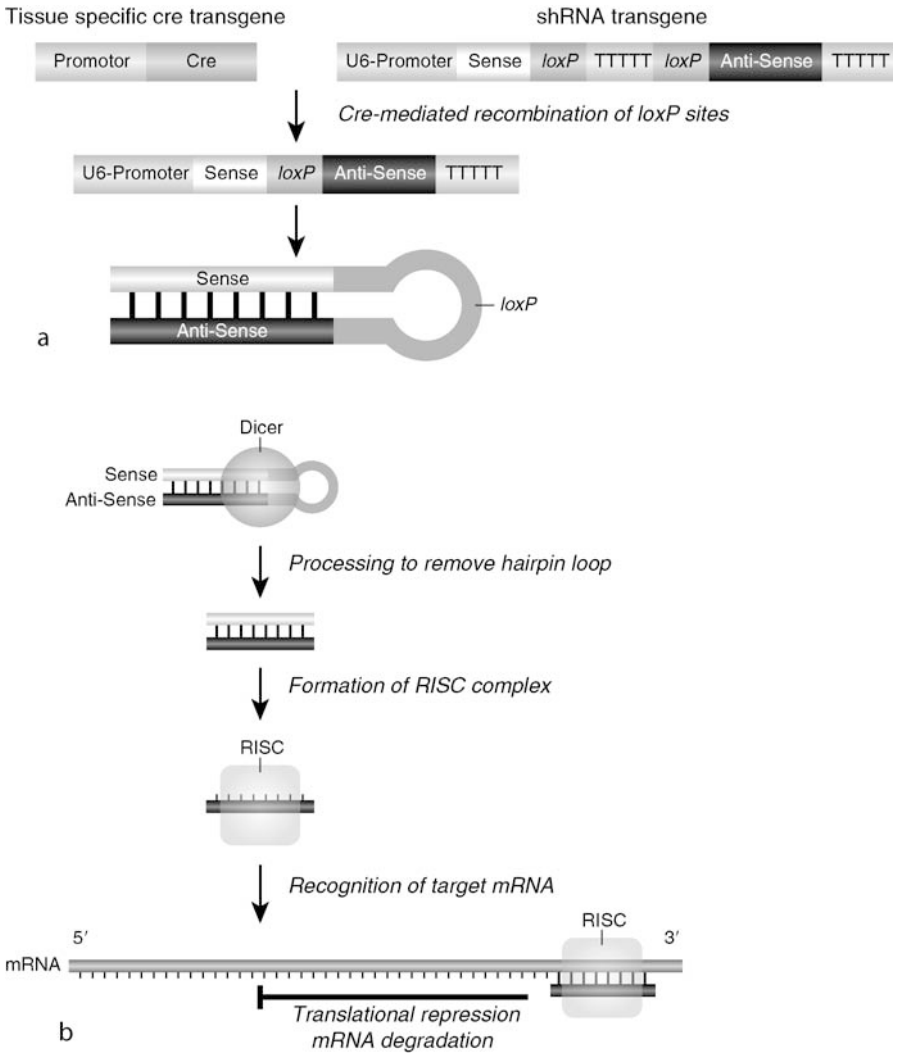
As mentioned above, many mutations that result in human disease are point mutations that result in "hypomorphic," or partial loss of function alleles of a gene. In this case a disease state may result from decreased activity of a gene product. In other cases, a point mutation or deletion mutation may produce a protein that interferes with the function of the normal gene; this is referred to as a dominant negative effect. This could occur in instances where a protein requires homodimerization for activity, and dimerization of a wild type and a mutant form of a protein leads to an inactive complex. Dominant-negative effects can also be found in cases where two proteins heterodimerize, and an inactive mutant protein is able to complex with its partner protein, but as before, the complex is inactive. Dominant – negative effects can be studied in animal models using transgenic mice. Although gene targeted mice discussed in the previous sections can also be considered to be transgenic, because foreign DNA is used to disrupt the endogenous gene, here the term "transgenic" is reserved for those mice in which foreign DNA has been inserted into the murine genome through pronuclear injection (42–45). In contrast to gene targeting schemes in which genes are modified in ES cells, and ES cells are then used to derive chimeric mice, transgenic strategies, DNA is directly microinjected into the pronucleus of a fertilized egg or zygote, and the injected zygotes are then reimplanted into the oviduct of a hormonally-primed female mouse. The injected DNA is able to recombine by non-homologous, or illegitimate recombination into random locations within the genome, and in variable amounts from zygote to zygote. Once mice are derived from the injected zygotes, they are tested to determine if they carry the injected DNA within their genomes as a transgene, and if they do, whether the transgene is expressed. By injecting DNA constructs that contain a tissue-specific promoter and a mutated gene of interest, it is possible to study whether expression of the mutant gene leads to an observable phenotype. In other instances, the gene to be expressed is not mutated, and the experiment is designed to determine if overexpression or de novo expression of the gene results in an observable phenotype or disease model. While the original transgenic studies used relatively short DNA constructs, more recent studies have used BAC (bacterial artificial chromsome) or YAC (yeast artificial chromosome)-based vectors whose much longer stretches of DNA containing promoters and other regulatory regions will hopefully confer more faithful patterns of expression of the transgene directed by those regulatory elements (42, 46). The great majority of transgenic work has been done in murine models, but there have been pioneering efforts in other species such as pigs and rats (47, 48).

Mutagenesis in Zebrafish

Possibly the single most important advancement in the development of novel animal models for kidney disease over the past 10 years is the establishment of zebrafish (*Danio rerio*) as a major model for understanding the genetic and physiological basis for disease. Zebrafish models of glomerular development and disease and polycystic kidney disease have been particularly useful models for study of human disease (49–55). Zebrafish are much less expensive to maintain than mice, and genetic tools to map zebrafish mutations nearly equal or in some cases extend beyond those available for mice (56–58). The zebrafish excretory system involves a pronephric duct and glomus that bears important similarity to mammalian nephrons, and has already been the subject of many research studies (49, 59–65). Two great advantages of Zebrafish are (1) their shorter

Figure 16-4

Conditional expression of shRNA transgenes. (a) Mice containing a Cre recombinase-expressing transgene and a shRNA transgene are crossed to obtain embryos or offspring mice containing both transgenes. The shRNA transgene contains a U6 promoter to direct RNA polymerase III mediated transcription, that is terminated by a poly-T sequence. It also contains a sense and anti-sense sequence, designed to target a mRNA, that will become the double stranded RNA that associates with the RISC complex to mediate the inhibitory effect on mRNA stability or translation. Therefore, transcription of the shRNA transgene is interrupted prior to Cre-mediated recombination of the first poly-T sequence. After removal of the first poly-T sequence by Cre-mediated recombination of the two LoxP sites, the transgene is fully transcribed and terminates at the second poly-T sequence. The resultant shRNA forms a hairpin loop by base pairing of the sense and ant-sense sequences. (b) The shRNA associates with DICER, an RNase III class enzyme that removes the hairpin loop. The double stranded RNA then associates with the RISC complex, the sense strand is removed, and the RISC complex with the anti-sense RNA finds its target mRNA and suppresses translation or decreases mRNA stability.



developmental timing, and (2) that development occurs in nearly transparent embryos that develop outside the mother, allowing for far greater observation and intervention than is possible with rodent embryos. The Zebrafish genome is presently being sequenced at the Sanger Center (66), and a full set of markers exists for gene mapping (57, 67, 68). Importantly, Zebrafish are not inbred like inbred mouse strains and there exists genetic

heterogeneity between isolates used in different laboratories. It is possible to make transgenic Zebrafish, though gene targeting is not yet possible (69–71). However, there have been large scale efforts to saturate the Zebrafish genome through insertional mutagenesis (72–74). An alternative to gene targeting is the use of morpholio oligonucleotides that inhibit expression of specific genes against which the morpholino is targeted; these can be injected into cells of early embryos, and phenotypes can be observed at various developmental stages thereafter (75, 76). In one sense, morpholinos have an advantage over gene knockouts, in that they can be designed to block expression of specific alternative splice forms (75), rather than all forms as is often the case with gene knockouts in mice.

Gene Identification- Phenotype to Genotype

Mutational Screens to Obtain New Phenotypes and Identify Genes

ENU mutagenesis: At present, several major efforts in several countries involve the use of N-ethyl-N-nitrosourea (ENU) to introduce small mutations throughout the mouse genome (77–82). Similar approaches have been used with Zebrafish and many interesting phenotypes have been obtained (83–87). These large genome-scale approaches, which can involve very large mouse or Zebrafish colonies, are justified by the following arguments: (1) Most disease-related human mutations are caused by point mutations, therefore a ENU-mutagenic approach may have a greater chance of producing a phenotype resembling a human disease than will gene-targeted mutations that usually completely inactivate a gene; (2) an ENU-based approach does not rely on previous identification or cloning of the gene, i.e., any gene is a theoretical target, and can be studied, to the extent that some degree of compromise in the gene product's activity will result in an observable phenotype. The obvious disadvantage in comparison with gene targeting is that a large amount of work lies between the observation of a phenotype and the final identification of the mutated gene. (3) Given a large enough effort, it should be possible to eventually "saturate" the genome with mutations, i.e., examination of several hundred thousand mutagen-treated mice or Zebrafish is likely to provide the opportunity to observe the effects of placing a mutation in every gene capable of causing an observable phenotype. However, one important point remains to be mentioned, that dramatically increases the labor and expense of an ENU-based effort. Most observable phenotypes tend to be genetically recessive instead of dominant, meaning that they are not apparent in the first generation offspring of mutagen-treated animals. Instead, it is necessary to breed a second generation and then backcross it to the first generation mice (or Zebrafish), resulting in a third generation (*Fig. 16-5*). Doing this on a large scale will result in many third generation animals that are now homozygous for mutations resulting from the original mutagenic treatment, and some will have observable phenotypes that can be studied for biological interest and to map the responsible gene.

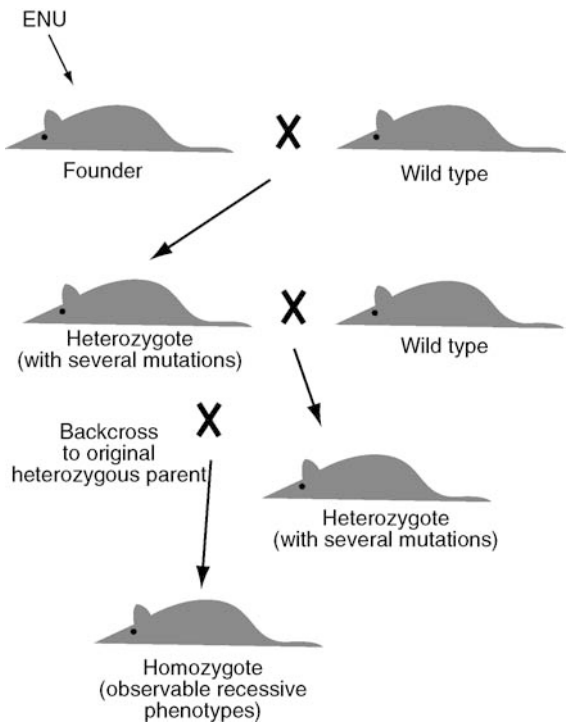
These large scale genetic approaches are not only suited to study developmental anomalies. Some of the large-scale efforts on mouse mutagenesis ongoing around the world will involve performing basic blood work and a urinalysis on each mouse from the group being screened for new phenotypes. Thus this approach has the potential to identify genes involved in disease progression, as well as those responsible for morphogenetic processes.

Gene Identification

Microsatellite repeats: Mapping sites of induced or spontaneous mutations in mice has been greatly aided by the development of sets of microsatellite repeat markers. Microsatellite repeats used in mapping are stretches of CA dinucleotide repeats that are found interspersed throughout mammalian genomes (88, 89). Typically, these CA repeats contain 10–20 CA dinucleotides. These CA repeats are flanked by unique sequences, and thus it is possible to design pairs of PCR primers that correspond to these flanking sequences, that will amplify the $(CA)_n$ sequence between the two primers. Within a genetically inbred strain of mice each individual mouse will contain the same number of CA dinucleotides at each repeat. However, similarly to the variation observed between human individuals, different inbred strains may differ in the number of CA dinucleotides at any particular repeat. Additionally, there are species of mice closely related to *mus musculus*, such as *mus spretus*, that provide even greater differences in the number of CA dinucleotides at many repeats than are found between the inbred strains of mus musculus. As depicted in *Fig. 16-6*, genetic mapping using microsatellite markers takes the following approach: A mouse (or mice) with a phenotype produced by induced or spontaneous mutagenesis is mated with a mouse from a different inbred strain, or from a different species, such as mus spretus to produce F1 mice that are now

Figure 16-5

ENU mutagenesis. A scheme is depicted for finding recessive phenotypes through ENU mutagenesis. Dominant phenotypes require a less complicated approach, as phenotypes will be apparent in the first generation derived from crossing founders with wild type mice. In this scheme, a mutagenized male founder that probably carries many mutations after mutagenesis, is mated with a wild type mouse to produce heterozygote offspring that carry a subset of these mutations. These heterozygotes are mated with wild type mice to produce a second generation, which will carry a smaller subset of the original mutations. These are then mated to the original heterozygous offspring of the founders, and 25% of the offspring of this cross will be homozygous for any particular mutation that was present in the second heterozygous generation.

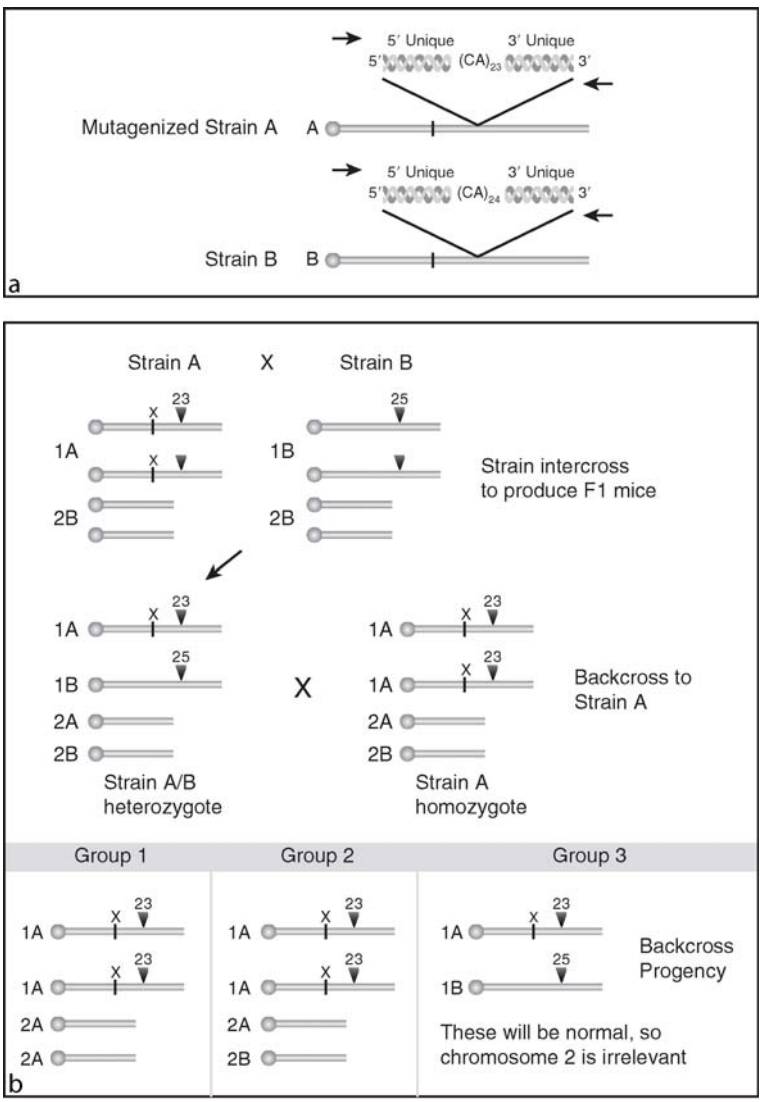


heterozygous at all loci, containing one allele from each of the two parental strains, and one mutant allele. In the case of a recessive phenotype, these F1 mice are now either backcrossed to the original mutant strain, or intercrossed among themselves, to produce approximately 100 progeny mice, about 25% of which can be expected to show the mutant phenotype. For dominant phenotypes, the backcross can be to a wild type mice of either parental strain, and 50% will display the phenotype. Importantly, during this back- or intercross, there is independent segregation of chromosomes, such that each individual of the 100 mice is genetically unique, in that at any given locus, it may be homozygous for alleles from a parental strain, or heterozygous, containing an allele from each strain. A set of PCR primers corresponding to about 40–50 microsatellite repeats (about 2–3 per mouse chromosome) are used in the first round of analysis. These are chosen such that the two parental strains are know to differ in the length of the CA repeat between each primer pair. DNA samples are now obtained from all the progeny, and are tested for the length of the CA sequence at each of the 40–50 microsatellites, and these results are correlated with the observed phenotypes (in practice, a computational result, called a LOD score is produced). Most of the microsatellites will not be genetically linked to the locus containing the mutation, and there will be no observable correlation between the strain genotype at a particular microsatellite and the presence or absence of the phenotype. In contrast, if a microsatellite marker is sufficiently closely linked to the site of a mutation causing a recessive phenotype, both alleles of the microsatellite marker are more likely to be derived from the parental strain originally containing the mutation. Thus, the goal of the first round of screening is to identify at least one marker that is linked to the mutation. Thereafter, subsequent rounds will use sets of markers linked to the original positive marker, with the expectation that it will be possible to identify a marker or pair of markers very closely linked to the mutation that will delimit the region of a single chromosome on which the mutation is located. This can then be used to initiate either a candidate gene approach or a chromosome walking approach to eventually identify the mutated gene.

SNPs and haplotype mapping: Newer more efficient approaches to mapping genetic elements are presently replacing the use of microsatellite repeats described in the previous section. Chief among these are the use of SNP microarrays. SNPs, or single nucleotide polymorphisms, are single base pair differences found between individuals within a species. Among the human population, SNPs tend to be found every 100–300 base pairs within the genome, and can be used as a measure of genetic relatedness among, for example, ethnic groups, or people in different geographic areas (90). SNPs are also found in comparing different inbred strains of mice and between the commonly used laboratory inbred strains that are all derived from *mus musculus* and other mouse strains that are "non-*musculus*" that can be intercrossed with mus musculus strains to aid in genetic mapping, as described above (91). In contrast, within a particular inbred strain of mice, there is by definition genetic homogeneity, and

Figure 16-6

A scheme for mapping a mutation to a genetic locus. (a) depicts the use of microsatellite CA repeats. Strains A and B are two inbred strains of mice, that differ in the length of many CA repeats, including the one shown here. Strain A has 23 CA dinucleotide repeats, whereas strain B has 25. They have the same unique 5′ and 3′ sequences flanking the CA repeats, thus the same PCR primers can be used for both strains, but amplification will yield a longer product from strain B than A. (b) The mating scheme to begin mapping the mutation. Only chromosome 1 and 2 are shown. Strain A has a homozygous mutation on chromosome 1, marked as an ''X,'' that is linked to the CA repeat, here designated by the black inverted triangle. This recessive mutation yields an observable phenotype. Strain A and B are mated to produce an F1 progeny, which will be heterozygous at all loci, including the one mutated in strain A. They will also be heterozygous for all CA repeats, including those on chromosome 2. Thus any CA repeats that differ between the two strains will yield two bands on a PCR reaction using the flanking unique primers for that CA repeat. F1 mice are backcrossed to Strain A homozygotes, and many offspring are examined. 50% of these offspring should be homozygous for X and have the observable phenotype. When these mice are analyzed for the CA repeat close to the locus for X, most mice with the phenotype will show only the strain A amplification product, whereas most of those without the phenotype will show both the strain A and strain B bands. In contrast, amplification of any CA repeats from chromosome 2 or any other chromosome, will not show any correlation of strain A homozyosity with the observed phenotype.



SNPs should not be present between individuals of the same strain. SNPs are also used to map genes in Zebrafish (68). See also *Fig. 16-7*.

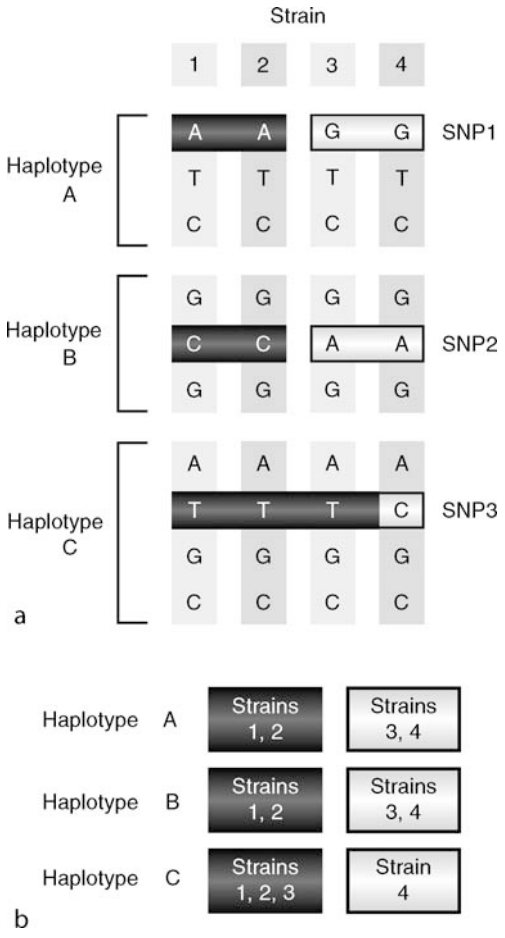
It is now possible to detect SNPs using microarrays (92, 93), such that thousands of oligonucleotides complementary to sequences containing known SNPs can be arrayed on a single microarray chip, and hybridized to an individual human or animal's DNA to determine which of thousands of particular SNPs that individual has in their genome (94). SNPs can be used as genetic markers similarly to the microsatellite repeats described in the previous section. Therefore, a single microarray can provide the same information as hundreds of PCR reactions. To be used in a genetic mapping experiment, the same type of inter-specific cross would be performed as described in the previous section, but instead of using PCR reactions to analyze the segregation of microsatellite repeats, a microarray is used for each individual to analyze the segregation of SNPs, to narrow the interval that contains the gene being mapped.

QTL and Haplotype Analysis

Almost all human disease has a genetic component, whether it is the relative susceptibility to an infectious agent, at one end of a spectrum, or a disease that is primarily due to a genetic mutation in a single gene, at the other end. Between these two extremes lies most human disease, whose etiology derives from a combination of genetic and environmental factors. In many instances, the genetic component is due to the effects of more than one gene. In other instances a single gene might be responsible, but the phenotype is not absolute, but rather of variable penetrance or severity. A genetic elements that contributes to a disease phenotype in a quantitative, as opposed to absolute, manner is referred to as a quantitative trait locus, or QTL (95). It is probably not an overstatement to say that most genetic components of disease occur as QTLs, and indeed, there are over 2,000 known QTLs that have been reported (96). Herein, however, lies the difficulty, as there are not standardized criteria for defining a QTL (95). Moreover, despite this enormous number of reported QTLs, only in the case of around 20–30 QTLs, depending on the criteria used to judge, have the responsible genes been identified and rigorously proven to be responsible for the disease phenotype (96). This is both due to the difficulty in identifying genes responsible for phenotypes in complex genomes, but also because in situations where a phenotype

Figure 16-7

SNPs and haplotypes. (a) A highly simplified of schematic of SNPs and haplotypes is depicted. Four mouse strains, labeled at top are compared. A three nucleotide stretch is shown for haplotypes A and B and four nucleotides for C, though in reality a haplotype may span megabases and be defined by hundreds or more SNPs. The sequences in haplotypes A, B and C are not necessarily contiguous and might even be on different chromosomes. In haplotype A, SNP1 is present at the first nucleotide, in haplotype B, SNP2 is present at the second nucleotide, and in haplotype C, SNP3 is present at the second nucleotide. (b) Haplotype grouping defined by the SNPs. For haplotypes A and B, strains 1 and 2 are the same haplotype, and 3 and 4 define a different haplotype. For haplotype C, strains 1, 2 and 3 are the same haplotype, and strain 4 is a different haplotype, suggesting that strain 3 might be more closely related to strains 1 and 2 than is strain 4. If strains 1 and 2 differ from 3 and 4 for a disease phenotype, it is more likely that the gene is within the area covered by haplotypes A and B, than within haplotype C, that is shared between strains 1, 2 and 3.



may be due to the combined actions of many genes, the contribution of any individual gene may be modest and difficult to prove in an experimental setting.

Nevertheless, many animal models of human disease appear to be caused by one or more QTLs, and the identification of the responsible gene(s) is a pursuit of many research laboratories. Importantly, comparisons of syntenic regions between human and rodent genomes has aided the search for genes at QTLs, as it is often apparent that a QTL identified in humans corresponds to a QTL for a similar phenotype in rodents (97). Comparisons of syntenic regions can also be complicated, as a region harboring a QTL in a rodent genome, if not already delimited to a narrow interval, may be syntenic with several regions of the human genome, that are found on different chromosomes.

The approach to identifying QTLs is similar to that used to detect monogenic phenotypes, that involved the detection of microsatellite repeats or SNPs described above. However, QTL analysis is much more complicated than the analysis of monogenic phenotypes, as one is likely to find multiple regions that appear to be linked to the phenotype, and no single region will stand out as the obvious candidate, until much larger numbers of individual animals or humans are analyzed to obtain statistically significant genetic data for each of several candidate genes.

Haplotype analysis has the potential to make gene identification by SNP mapping more efficient (97–99). Haplotypes are defined as a group of genetic markers that are physically linked on chromosomes and that tend to be inherited together more often than might be predicted if genetic recombination events were evenly distributed along chromosomes. Perhaps the best know examples are the major histocompatibility loci, HLA in humans and H2 in mice. It is now known that haplotype units exist throughout mammalian genomes, and that SNPs can be used as markers to define haplotypes (97–99). It then follows that inbred mouse strains that more closely genetically related are likely to have the same haplotype at a particular genomic location, whereas more distantly related strains would be more likely to have different haplotypes (97, 98). Thus, when SNPs are being analyzed in the progeny of a genetic cross in an attempt to narrow down the location of a candidate gene, haplotype analysis provides an alternative to considering each SNP individually. For example, if strains A and B are being studied with the aim of identifying a disease related QTL only present in strain A and strain B being normal, then genomic areas where strains A and B share the same haplotype are unlikely to harbor the disease locus, whereas areas of the genome where they have differing haplotypes are more likely to have the disease locus. Therefore, consideration of haplotypes, each of which may contain hundreds of SNPs, may allow a means of focusing on candidate genes within differing haplotypes, and ignoring those located within regions where the affected and unaffected strains share the same haplotype, that might otherwise be suggested for further study through a SNP analysis that did not take haplotypes into account. In practice, such an analysis may involve multiple strains of mice and crosses between them in an effort to narrow a genetic interval and physically locate a QTL (100, 101).

Implications of Genome Sequencing

Sequencing of the entire human genome was completed in 2001 (102, 103) and of the mouse genome in 2002 (104). These continue to be refined, with more detailed reports and annotations of the sequence of specific human chromosomes published from 2003 to 2006. As of this writing, the sequences of approximately 180 organisms, including bacteria, plants and animals have been reported. This rapidly expanding database has transformed modern biology and the study of disease, and newer high throughput sequencing technologies promise an even more rapid expansion in of sequence data in the near future (105). For example, in gene mapping studies described above, it is no longer the situation that a disease gene might be mapped to an area within a chromosome that had never been sequenced, and the hunt for the gene becomes a large scale sequencing project. Now, once an area is delimited, the candidate genes in that area are largely known from prior genome sequencing, allowing much more directed sequencing to be done in attempts to find disease-causing mutations. The genome sequences for most, if not all, animals used as disease models is also known, greatly accelerating studies such as those involving disease-related gene identification in animal models. A major frontier in genome sequencing related to disease models is not so much the sequencing of additional species as it is the sequencing of multiple genomes within a species, so that we can increase our understanding of intra-species genetic variation, and how it relates to disease susceptibility (105–107).

Other Animals in Nephrology Research

The emphasis on mice and Zebrafish thus far in this chapter is not meant to overlook the enormous benefit that has derived from the use of other species. Despite

some pioneering efforts, gene targeting technology has not been developed in rats. However, ENU mutagenesis has been used to obtain rat mutant models of disease and gene mapping of disease phenotypes in rats has yielded important insights (108–112). Rats have also been widely used in studies of nutrition as it related to kidney development and disease (113). Furthermore, their larger size makes rats more amenable to studies that require precise physiological monitoring or imaging, though the technology to perform such studies in murine models, to take advantage of knockout mouse models, has also improved dramatically (114–121).

Large animal research have also had an important legacy in nephrology that continues to this day. Historically, several animal models have been used to study renal physiology, including swine, sheep, guinea pigs, rabbits, rats and mice. Fetal lambs have been a particularly important model in which developmental aspects of physiology have been studied, particularly relating to obstructive uropathy (122–134). Studies in large animals are also vital in efforts to use tissue engineering to develop artificial tissues or in models of tissue regeneration (135–137). As in other situations, there is a constant need to balance the advantages of a large animal model with the lower costs of smaller animals models.

Models of Renal Failure

Approaches to the study of renal failure include acute and chronic models. Acute renal failure has been induced using pharmacological agents, antisera against kidney tissue or other antigens in which immune complex formation leads to glomerular disease (138–144). Ischemia-reperfusion models of acute renal failure are achieved by temporarily ligating a renal artery allowing study of the pathological processes involved in tubular damage, as well as the effect of various pharmacological treatments on the pathologic process (e.g., (145–165)). An alternate form of tubular injury involves temporary obstruction of a ureter (166–168). Combining these injury models with genetic models is an emerging frontier in nephrology research.

Models of Immunological Injury

There are many models of autoimmune injury to the kidney. A traditional model for a lupus-like autoimmune disease is the NZB mouse, that has been studied for many years (169–173). These mice develop autoantibodies similar to those observed in humans with systemic lupus erythematosis and other related autoimmune disorders. More recently, many strains of mice carrying mutations in genes involved in the regulation of the immune response have been used to increase our understanding of the role the immune system plays in the onset and progression of kidney disease (174–184). These knockout strains have allowed investigators to begin a genetic dissection of genes involved in autoimmune and other disorders. Transgenic technologies have also had an important impact in the development of new immunological models. One particular contribution is the use of live-cell imaging that exploits fluorescent transgenic reporter genes which mimic the expression of genes expressed in specific immunological cell types (185, 186). These can be used either to trace the location of these cells in animals, or to show evidence of gene expression in an in vivo setting.

Transplant Models

Animal models have been used extensively to study transplant rejection, and in efforts to understand how tolerance to transplanted tissue may be improved. Over the past 20 years there has been an extraordinary advancement in our mechanistic understanding of the immune function, and this has been brought to bear on the study of transplant rejection and tolerance (187–193). Important models under study include skin and heart transplants in mice, and kidney transplants in rats (194–201). Additionally, it is possible to produce "humanized" mice by transplanting human tissue into immunodeficient or irradiated mice whose immune system has been reconstituted with human lymphocytes, thus allowing the study of human immune function in an animal model (202–205). One area of research that remains controversial is that involving xenografts (206, 207). Since the supply of human kidneys and other organs for transplants continues to fall far short of the demand, there is a desirability of determining whether non-human animals provide an alternative source of organs for transplantation. The major concerns here include the strong immunological rejection to a xenograft that must be overcome, and the danger that xenografts might serve as vectors for the introduction of novel infectious agents into the human population.

Summary

Animal models are of increasing importance in the study of kidney disease. An important shift over the past 10 or

more years is the use of rodent models, and the use of genetic models. Large animal use remains an important aspect of these studies. The use of large versus small animals must take into account cost and ethical considerations.

References

1. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R. WT-1 is required for early kidney development. *Cell* 1993;74(4):679–691.
2. Torres M, Gomez PE, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. *Development* 1995;121(12): 4057–4065.
3. Moore MW, Klein RD, Farinas I, Sauer H, Armani M, Philips H, Reichardt LF, Ryan AM, Carver-Moore K, Rosenthal A. Renal and neuronal abnormalities in mice lacking GDNF. *Nature* 1996; 382:76–79.
4. Pichel JG, Shen L, Sheng HZ, Granholm A-C, Drago J, Grinberg A, Lee EJ, Huang SP, Saarma M, Hoffer BJ, Sariola H, Westphal H. Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* 1996;382:73–76.
5. Sanchez MP, Silos-Santiago I, Frisen J, He B, Lira SA, Barbacid M. Renal agenesis and absence of enteric ganglions in mice lacking GDNF. *Nature* 1996;382:70–74.
6. Kispert A, Vainio S, McMahon AP. Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. *Development* 1998;125(21):4225–4234.
7. Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev* 1995. 9(22): 2795–807.
8. Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev* 1995;9(22): 2808–2820.
9. Robertson EJ. Isolation of embryonic stem cells. In Teratocarcinomas and Embryonic Stem Cells: A Practical Approach. Robertson EJ (ed.) Oxford, IRL Press, 1987, pp. 71–112.
10. Bradley A. Production and analysis of chimeric mice. In Teratocarcinomas and Embryonic Stem Cells: A Practical Approach. Robertson EJ (ed.) Oxford, IRL Press, 1987, pp. 113–151.
11. Thomas KR, Capecchi MR. Targeting of genes to specific sites in the mammalian genome. *Cold Spring Harb Symp Quant Biol* 1986;51(1):1101–1113.
12. Thomas KR, Deng C, Capecchi MR. High-fidelity gene targeting in embryonic stem cells by using sequence replacement vectors. *Mol Cell Biol* 1992;12(7):2919–2923.
13. Capecchi MR. The new mouse genetics: altering the genome by gene targeting. *Trends Genet* 1989;5(3):70–76.
14. Orban PC, Chui D, Marth JD. Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci USA* 1992;89 (15):6861–6865.
15. Sauer B. Inducible gene targeting in mice using the Cre/lox system. *Methods* 1998;14(4):381–392.
16. Stricklett PK, Nelson RD, Kohan DE. The Cre/loxP system and gene targeting in the kidney. *Am J Physiol* 1999;276(5 Pt 2):F651–F657.
17. Furth PA, St. Onge L, Boger H, Gruss P, Gossen M, Kistner A, Bujard H, Hennighausen L. Temporal control of gene expression in transgenic mice by a tetracycline responsive promoter. *PNAS* 1994;91:9302–9306.
18. Sohal DS, Nghiem M, Crackower MA, Witt SA, Kimball TR, Tymitz KM, Penninger JM, Molkentin JD. Temporally regulated and tissue-specific gene manipulations in the adult and embryonic heart using a tamoxifen-inducible Cre protein. *Circ Res* 2001;89(1):20–25.
19. Verrou C, Zhang Y, Zurn C, Schamel WW, Reth M. Comparison of the tamoxifen regulated chimeric Cre recombinases MerCreMer and CreMer. *Biol Chem* 1999;380(12):1435–1438.
20. Gawlik A, Quaggin SE. Conditional gene targeting in the kidney. *Curr Mol Med* 2005;5(5):527–536.
21. Park JS, Valerius MT, McMahon AP. Wnt/beta-catenin signaling regulates nephron induction during mouse kidney development. *Development* 2007;134(13):2533–2539.
22. Moeller MJ, Sanden SK, Soofi A, Wiggins RC, Holzman LB. Two gene fragments that direct podocyte-specific expression in transgenic mice. *J Am Soc Nephrol* 2002;13(6):1561–1567.
23. Wong MA, Cui S, Quaggin SE. Identification and characterization of a glomerular-specific promoter from the human nephrin gene. *Am J Physiol Renal Physiol* 2000;279:F1027–F1032.
24. Eremina V, Wong MA, Cui S, Schwartz L, Quaggin SE. Glomerular-specific gene excision in vivo. *J Am Soc Nephrol* 2002;13(3):788–793.
25. Li H, Zhou X, Davis DR, Xu D, Sigmund CD. An androgen-inducible proximal tubule-specific Cre recombinase transgenic model. *Am J Physiol Renal Physiol* 2008;294(6):F1481–F1486.
26. Dworniczak B, Skryabin B, Tchinda J, Heuck S, Seesing FJ, Metzger D, Chambon P, Horst J, Pennekamp P. Inducible Cre/loxP recombination in the mouse proximal tubule. *Nephron Exp Nephrol* 2007;106 (1):e11–20.
27. Rubera I, Poujeol C, Bertin G, Hasseine L, Counillon L, Poujeol P, Tauc M. Specific Cre/Lox recombination in the mouse proximal tubule. *J Am Soc Nephrol* 2004;15(8):2050–2056.
28. Stricklett PK, Taylor D, Nelson RD, Kohan DE. Thick ascending limb-specific expression of Cre recombinase. *Am J Physiol Renal Physiol* 2003;285(1):F33–F39.
29. Marose TD, Merkel CE, McMahon AP, Carroll TJ. Beta-catenin is necessary to keep cells of ureteric bud/Wolffian duct epithelium in a precursor state. *Dev Biol* 2008;314(1):112–126.
30. Stricklett PK, Nelson RD, Kohan DE. Targeting collecting tubules using the aquaporin-2 promoter. *Exp Nephrol* 1999;7(1):67–74.
31. Li WL, Cheng X, Tan XH, Zhang JS, Sun YS, Chen L, Yang X. Endothelial cell-specific expression of Cre recombinase in transgenic mice. *Yi Chuan Xue Bao* 2005;32(9):909–915.
32. Licht AH, Raab S, Hofmann U, Breier G. Endothelium-specific Cre recombinase activity in flk-1-Cre transgenic mice. *Dev Dyn* 2004;229 (2):312–318.
33. Cattelino A, Liebner S, Gallini R, Zanetti A, Balconi G, Corsi A, Bianco P, Wolburg H, Moore R, Oreda B, Kemler R, Dejana E. The conditional inactivation of the beta-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility. *J Cell Biol* 2003;162(6):1111–1122.
34. Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. Simple and highly efficient BAC recombineering using galK selection. *Nucleic Acids Res* 2005;33(4):e36.
35. Zhang XM, Huang JD. Combination of overlapping bacterial artificial chromosomes by a two-step recombinogenic engineering method. *Nucleic Acids Res* 2003;31(15):e81.
36. Testa G, Zhang Y, Vintersten K, Benes V, Pijnappel WW, Chambers I, Smith AJ, Smith AG, Stewart AF. Engineering the mouse genome with bacterial artificial chromosomes to create multipurpose alleles. *Nat Biotechnol* 2003;21(4):443–447.

37. Cheng JC, Moore TB, Sakamoto KM. RNA interference and human disease. *Mol Genet Metab* 2003;80(1–2):121–128.
38. Campbell TN, Choy FY. RNA interference: past, present and future. *Curr Issues Mol Biol* 2005;7(1):1–6.
39. Tijsterman M, Plasterk RH. Dicers at RISC; the mechanism of RNAi. *Cell* 2004;117(1):1–3.
40. Shukla V, Coumoul X, Deng CX. RNAi-based conditional gene knockdown in mice using a U6 promoter driven vector. *Int J Biol Sci* 2007;3(2):91–99.
41. Coumoul X, Deng CX. RNAi in mice: a promising approach to decipher gene functions in vivo. *Biochimie* 2006;88(6):637–643.
42. Vintersten K, Testa G, Naumann R, Anastassiadis K, Stewart AF. Bacterial artificial chromosome transgenesis through pronuclear injection of fertilized mouse oocytes. *Methods Mol Biol* 2008;415:83–100.
43. Feng G, Lu J, Gross J. Generation of transgenic mice. *Methods Mol Med* 2004;99:255–267.
44. Isola LM, Gordon JW. Transgenic animals: a new era in developmental biology and medicine. *Biotechnology* 1991;16:3–20.
45. Gordon JW, Ruddle FH. Gene transfer into mouse embryos: production of transgenic mice by pronuclear injection. *Methods Enzymol* 1983;101:411–433.
46. Schedl A, Larin Z, Montoliu L, Thies E, Kelsey G, Lehrach H, Schutz G. A method for the generation of YAC transgenic mice by pronuclear microinjection. *Nucleic Acids Res* 1993;21 (20):4783–4787.
47. Nottle MB, Nagashima H, Verma PJ, Du ZT, Grupen CG, Ashman RJ, MacIlfatrick S. Developments in transgenic techniques in pigs. *J Reprod Fertil Suppl* 1997;52:237–244.
48. Filipiak WE, Saunders TL. Advances in transgenic rat production. *Transgenic Res* 2006;15(6):673–686.
49. Majumdar A, Drummond IA. Podocyte differentiation in the absence of endothelial cells as revealed in the zebrafish avascular mutant, cloche. *Dev Genet* 1999;24(3–4):220–229.
50. Drummond I. Making a zebrafish kidney: a tale of two tubes. *Trends Cell Biol* 2003;13(7):357–365.
51. Hostetter CL, Sullivan-Brown JL, Burdine RD. Zebrafish pronephros: a model for understanding cystic kidney disease. *Dev Dyn* 2003;228(3):514–522.
52. Drummond IA. Zebrafish kidney development. *Methods Cell Biol* 2004;76:501–530.
53. Sun Z, Amsterdam A, Pazour GJ, Cole DG, Miller MS, Hopkins N. A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney. *Development* 2004;131(16):4085–4093.
54. Hentschel DM, Park KM, Cilenti L, Zervos AS, Drummond I, Bonventre JV. Acute renal failure in zebrafish: a novel system to study a complex disease. *Am J Physiol Renal Physiol* 2005;288(5): F923–F929.
55. Hentschel DM, Mengel M, Boehme L, Liebsch F, Albertin C, Bonventre JV, Haller H, Schiffer M. Rapid screening of glomerular slit diaphragm integrity in larval zebrafish. *Am J Physiol Renal Physiol* 2007;293(5):F1746–F1750.
56. Fishman MC. Zebrafish genetics: the enigma of arrival. *Proc Natl Acad Sci USA* 1999;96(19):10554–10556.
57. Shimoda N, Knapik EW, Ziniti J, Sim C, Yamada E, Kaplan S, Jackson D, de Sauvage F, Jacob H, Fishman MC. Zebrafish genetic map with 2000 microsatellite markers. *Genomics* 1999;58(3):219–232.
58. Knapik EW, Goodman A, Ekker M, Chevrette M, Delgado J, Neuhauss S, Shimoda N, Driever W, Fishman MC, Jacob HJ. A microsatellite genetic linkage map for zebrafish (Danio rerio). *Nat Genet* 1998;18(4):338–343.
59. Drummond IA, Majumdar A, Hentschel H, Elger M, Solnica-Krezel L, Schier AF, Neuhauss SC, Stemple DL, Zwartkruis F, Rangini Z, Driever W, Fishman MC. Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. *Development* 1998;125(23):4655–4667.
60. Liu S, Lu W, Obara T, Kuida S, Lehoczky J, Dewar K, Drummond IA, Beier DR. A defect in a novel Nek-family kinase causes cystic kidney disease in the mouse and in zebrafish. *Development* 2002;129 (24):5839–5846.
61. Briggs JP. The zebrafish: a new model organism for integrative physiology. *Am J Physiol Regul Integr Comp Physiol* 2002;282(1): R3–9.
62. Serluca FC, Fishman MC. Pre-pattern in the pronephric kidney field of zebrafish. *Development* 2001;128(12):2233–2241.
63. Majumdar A, Drummond IA. The zebrafish floating head mutant demonstrates podocytes play an important role in directing glomerular differentiation. *Dev Biol* 2000;222(1):147–157.
64. Drummond IA. The zebrafish pronephros: a genetic system for studies of kidney development. *Pediatr Nephrol* 2000;14(5): 428–435.
65. Majumdar A, Lun K, Brand M, Drummond IA. Zebrafish no isthmus reveals a role for pax2.1 in tubule differentiation and patterning events in the pronephric primordia. *Development* 2000;127 (10):2089–2098.
66. Vogel G. GENOMICS: Sanger will sequence zebrafish genome. *Science* 2000;290(5497):1671b.
67. Stickney HL, Schmutz J, Woods IG, Holtzer CC, Dickson MC, Kelly PD, Myers RM, Talbot WS. Rapid mapping of zebrafish mutations with SNPs and oligonucleotide microarrays. *Genome Res* 2002;12 (12):1929–1934.
68. Bradley KM, Elmore JB, Breyer JP, Yaspan BL, Jessen JR, Knapik EW, Smith JR. A major zebrafish polymorphism resource for genetic mapping. *Genome Biol* 2007;8(4):R55.
69. Damert A, Kusserow H. Generation of transgenic mice by pronuclear injection. *Methods Mol Med* 2003;89:513–528.
70. Gaiano N, Allende M, Amsterdam A, Kawakami K, Hopkins N. Highly efficient germ-line transmission of proviral insertions in zebrafish. *Proc Natl Acad Sci USA* 1996;93(15):7777–7782.
71. Gaiano N, Hopkins N. Introducing genes into zebrafish. *Biochim Biophys Acta* 1996;1288(1):O11–14.
72. Amsterdam A, Hopkins N. Retrovirus-mediated insertional mutagenesis in zebrafish. *Methods Cell Biol* 1999;60:87–98.
73. Talbot WS, Hopkins N. Zebrafish mutations and functional analysis of the vertebrate genome. *Genes Dev* 2000;14(7):755–762.
74. Amsterdam A, Hopkins N. Mutagenesis strategies in zebrafish for identifying genes involved in development and disease. *Trends Genet* 2006;22(9):473–478.
75. Draper BW, Morcos PA, Kimmel CB. Inhibition of zebrafish fgf8 premRNA splicing with morpholino oligos: a quantifiable method for gene knockdown. *Genesis* 2001;30(3):154–156.
76. Scholpp S, Brand M. Morpholino-induced knockdown of zebrafish engrailed genes eng2 and eng3 reveals redundant and unique functions in midbrain–hindbrain boundary development. *Genesis* 2001;30(3):129–133.
77. Hrabe de Angelis M, Strivens M. Large-scale production of mouse phenotypes: the search for animal models for inherited diseases in humans. *Brief Bioinform* 2001;2(2):170–180.
78. Nolan PM, Peters J, Vizor L, Strivens M, Washbourne R, Hough T, Wells C, Glenister P, Thornton C, Martin J, Fisher E, Rogers D, Hagan J, Reavill C, Gray I, Wood J, Spurr N, Browne M, Rastan S, Hunter J, Brown SD. Implementation of a large-scale ENU

mutagenesis program: towards increasing the mouse mutant resource. *Mamm Genome* 2000;11(7):500–506.
79. Chen Y, Yee D, Dains K, Chatterjee A, Cavalcoli J, Schneider E, Om J, Woychik RP, Magnuson T. Genotype-based screen for ENU-induced mutations in mouse embryonic stem cells. *Nat Genet* 2000;24 (3):314–317.
80. Anderson KV. Finding the genes that direct mammalian development: ENU mutagenesis in the mouse. *Trends Genet* 2000;16 (3):99–102.
81. Justice MJ, Noveroske JK, Weber JS, Zheng B, Bradley A. Mouse ENU mutagenesis. *Hum Mol Genet* 1999;8(10):1955–1963.
82. Hrabe de Angelis M, Balling R. Large scale ENU screens in the mouse: genetics meets genomics. *Mutat Res* 1998;400 (1–2):25–32.
83. Knapik EW. ENU mutagenesis in zebrafish–from genes to complex diseases. *Mamm Genome* 2000;11(7):511–519.
84. Beckwith LG, Moore JL, Tsao-Wu GS, Harshbarger JC, Cheng KC. Ethylnitrosourea induces neoplasia in zebrafish (Danio rerio). *Lab Invest* 2000;80(3):379–385.
85. Weinstein BM, Schier AF, Abdelilah S, Malicki J, Solnica-Krezel L, Stemple DL, Stainier DY, Zwartkruis F, Driever W, Fishman MC. Hematopoietic mutations in the zebrafish. *Development* 1996;123:303–309.
86. Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, van Eeden FJ, Jiang YJ, Heisenberg CP, Kelsh RN, Furutani-Seiki M, Vogelsang E, Beuchle D, Schach U, Fabian C, Nusslein-Volhard C. The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. *Development* 1996;123:1–36.
87. Solnica-Krezel L, Schier AF, Driever W. Efficient recovery of ENU-induced mutations from the zebrafish germline. *Genetics* 1994;136 (4):1401–1420.
88. Dietrich WF, Copeland NG, Gilbert DJ, Miller JC, Jenkins NA, Lander ES. Mapping the mouse genome: current status and future prospects. *Proc Natl Acad Sci USA* 1995;92(24):10849–10853.
89. Brown DM, Matise TC, Koike G, Simon JS, Winer ES, Zangen S, McLaughlin MG, Shiozawa M, Atkinson OS, Hudson JR Jr., Chakravarti A, Lander ES, Jacob HJ. An integrated genetic linkage map of the laboratory rat. *Mamm Genome* 1998;9(7):521–530.
90. Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M. Lander ES. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 1998;280(5366):1077–1082.
91. Tsang S, Sun Z, Luke B, Stewart C, Lum N, Gregory M, Wu X, Subleski M, Jenkins NA, Copeland NG, Munroe DJ. A comprehensive SNP-based genetic analysis of inbred mouse strains. *Mamm Genome* 2005;16(7):476–480.
92. Grant SF, Hakonarson H. Microarray technology and applications in the arena of genome-wide association. *Clin Chem* 2008;54 (7):1116–1124.
93. Patil N, Nouri N, McAllister L, Matsukaki H, Ryder T. Single-nucleotide polymorphism genotyping using microarrays. *Curr Protoc Hum Genet* 2001. Chapter 2: Unit 29.
94. Pletcher MT, McClurg P, Batalov S, Su AI, Barnes SW, Lagler E, Korstanje R, Wang X, Nusskern D, Bogue MA, Mural RJ, Paigen B, Wiltshire T. Use of a dense single nucleotide polymorphism map for in silico mapping in the mouse. *PLoS Biol* 2004;2(12):e393.
95. Abiola O, Angel JM, Avner P, Bachmanov AA, Belknap JK, Bennett B, Blankenhorn EP, Blizard DA, Bolivar V, Brockmann GA, Buck KJ, Bureau JF, Casley WL, Chesler EJ, Cheverud JM, Churchill GA, Cook M, Crabbe JC, Crusio WE, Darvasi A, de Haan G, Dermant P, Doerge RW, Elliot RW, Farber CR, Flaherty L, Flint J, Gershenfeld H, Gibson JP, Gu J, Gu W, Himmelbauer H, Hitzemann R, Hsu HC, Hunter K, Iraqi FF, Jansen RC, Johnson TE, Jones BC, Kempermann G, Lammert F, Lu L, Manly KF, Matthews DB, Medrano JF, Mehrabian M, Mittlemann G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Mountz JD, Nagase H, Nowakowski RS, O'Hara BF, Osadchuk AV, Paigen B, Palmer AA, Peirce JL, Pomp D, Rosemann M, Rosen GD, Schalkwyk LC, Seltzer Z, Settle S, Shimomura K, Shou S, Sikela JM, Siracusa LD, Spearow JL, Teuscher C, Threadgill DW, Toth LA, Toye AA, Vadasz C, Van Zant G, Wakeland E, Williams RW, Zhang HG, Zou F. The nature and identification of quantitative trait loci: a community's view. *Nat Rev Genet* 2003;4 (11):911–916.
96. Korstanje R, Paigen B. From QTL to gene: the harvest begins. *Nat Genet* 2002;31(3):235–236.
97. DiPetrillo K, Wang X, Stylianou IM, Paigen B. Bioinformatics toolbox for narrowing rodent quantitative trait loci. *Trends Genet* 2005;21(12):683–692.
98. Cuppen E. Haplotype-based genetics in mice and rats. *Trends Genet* 2005;21(6):318–322.
99. Guryev V, Smits BM, van de Belt J, Verheul M, Hubner N, Cuppen E. Haplotype block structure is conserved across mammals. *PLoS Genet* 2006;2(7):1767–1772.
100. Wang X, Korstanje R, Higgins D, Paigen B. Haplotype analysis in multiple crosses to identify a QTL gene. *Genome Res* 2004;14 (9):1767–1772.
101. Wittenburg H, Lyons MA, Li R, Kurtz U, Wang X, Mossner J, Churchill GA, Carey MC, Paigen B. QTL mapping for genetic determinants of lipoprotein cholesterol levels in combined crosses of inbred mouse strains. *J Lipid Res* 2006;47(8):1780–1790.
102. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K,

Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ. Initial sequencing and analysis of the human genome. *Nature* 2001;409(6822):860–921.

103. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. *Science* 2001;291(5507):1304–1351.

104. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, Antonarakis SE, Attwood J, Baertsch R, Bailey J, Barlow K, Beck S, Berry E, Birren B, Bloom T, Bork P, Botcherby M, Bray N, Brent MR, Brown DG, Brown SD, Bult C, Burton J, Butler J, Campbell RD, Carninci P, Cawley S, Chiaromonte F, Chinwalla AT, Church DM, Clamp M, Clee C, Collins FS, Cook LL, Copley RR, Coulson A, Couronne O, Cuff J, Curwen V, Cutts T, Daly M, David R, Davies J, Delehaunty KD, Deri J, Dermitzakis ET, Dewey C, Dickens NJ, Diekhans M, Dodge S, Dubchak I, Dunn DM, Eddy SR, Elnitski L, Emes RD, Eswara P, Eyras E, Felsenfeld A, Fewell GA, Flicek P, Foley K, Frankel WN, Fulton LA, Fulton RS, Furey TS, Gage D, Gibbs RA, Glusman G, Gnerre S, Goldman N, Goodstadt L, Grafham D, Graves TA, Green ED, Gregory S, Guigo R, Guyer M, Hardison RC, Haussler D, Hayashizaki Y, Hillier LW, Hinrichs A, Hlavina W, Holzer T, Hsu F, Hua A, Hubbard T, Hunt A, Jackson I, Jaffe DB, Johnson LS, Jones M, Jones TA, Joy A, Kamal M, Karlsson EK, Karolchik D, Kasprzyk A, Kawai J, Keibler E, Kells C, Kent WJ, Kirby A, Kolbe DL, Korf I, Kucherlapati RS, Kulbokas EJ, Kulp D, Landers T, Leger JP, Leonard S, Letunic I, Levine R, Li J, Li M, Lloyd C, Lucas S, Ma B, Maglott DR, Mardis ER, Matthews L, Mauceli E, Mayer JH, McCarthy M, McCombie WR, McLaren S, McLay K, McPherson JD, Meldrim J, Meredith B, Mesirov JP, Miller W, Miner TL, Mongin E, Montgomery KT, Morgan M, Mott R, Mullikin JC, Muzny DM, Nash WE, Nelson JO, Nhan MN, Nicol R, Ning Z, Nusbaum C, O'Connor MJ, Okazaki Y, Oliver K, Overton-Larty E, Pachter L, Parra G, Pepin KH, Peterson J, Pevzner P, Plumb R, Pohl CS, Poliakov A, Ponce TC, Ponting CP, Potter S, Quail M, Reymond A, Roe BA, Roskin KM, Rubin EM, Rust AG, Santos R, Sapojnikov V, Schultz B, Schultz J, Schwartz MS, Schwartz S, Scott C, Seaman S, Searle S, Sharpe T, Sheridan A, Shownkeen R, Sims S, Singer JB, Slater G, Smit A, Smith DR, Spencer B, Stabenau A, Stange-Thomann N, Sugnet C, Suyama M, Tesler G, Thompson J, Torrents D, Trevaskis E, Tromp J, Ucla C, Ureta-Vidal A, Vinson JP, Von Niederhausern AC, Wade CM, Wall M, Weber RJ, Weiss RB, Wendl MC, West AP, Wetterstrand K, Wheeler R, Whelan S, Wierzbowski J, Willey D, Williams S, Wilson RK, Winter E, Worley KC, Wyman D, Yang S, Yang SP, Zdobnov EM, Zody MC, Lander ES. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002;420(6915):520–562.

105. Bentley DR. Whole-genome re-sequencing. *Curr Opin Genet Dev* 2006;16(6):545–552.

106. Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Margulies M, Weinstock GM, Gibbs RA, Rothberg JM. The complete genome of an individual by massively parallel DNA sequencing. *Nature* 2008;452(7189):872–876.

107. Raven P, Fauquet C, Swaminathan MS, Borlaug N, Samper C. Where next for genome sequencing? *Science* 2006;311(5760):468.

108. Shiozawa M, Provoost AP, van Dokkum RP, Majewski RR, Jacob HJ. Evidence of gene-gene interactions in the genetic susceptibility to renal impairment after unilateral nephrectomy. *J Am Soc Nephrol* 2000;11(11):2068–2078.

109. Kwitek-Black AE, Jacob HJ. The use of designer rats in the genetic dissection of hypertension. *Curr Hypertens Rep* 2001;3(1):12–18.

110. Stoll M, Jacob HJ. Genetic rat models of hypertension: relationship to human hypertension. *Curr Hypertens Rep* 2001;3(2):157–164.

111. Stoll M, Cowley AW Jr., Tonellato PJ, Greene AS, Kaldunski ML, Roman RJ, Dumas P, Schork NJ, Wang Z, Jacob HJ. A genomic-systems biology map for cardiovascular function. *Science* 2001;294 (5547):1723–1726.
112. Jacob HJ, Kwitek AE. Rat genetics: attaching physiology and pharmacology to the genome. *Nat Rev Genet* 2002;3(1):33–42.
113. Rao GN. Diet and kidney diseases in rats. *Toxicol Pathol* 2002;30 (6):651–656.
114. Ma X, Abboud FM, Chapleau MW. Analysis of afferent, central, and efferent components of the baroreceptor reflex in mice. *Am J Physiol Regul Integr Comp Physiol* 2002;283(5):R1033–1040.
115. Ishii T, Kuwaki T, Masuda Y, Fukuda Y. Postnatal development of blood pressure and baroreflex in mice. *Auton Neurosci* 2001;94 (1–2):34–41.
116. Gross V, Plehm R, Tank J, Jordan J, Diedrich A, Obst M, Luft FC. Heart rate variability and baroreflex function in AT2 receptor-disrupted mice. *Hypertension* 2002;40(2):207–213.
117. Rokosh DG, Simpson PC. Knockout of the alpha 1A/C-adrenergic receptor subtype: the alpha 1A/C is expressed in resistance arteries and is required to maintain arterial blood pressure. *Proc Natl Acad Sci USA* 2002;99(14):9474–9479.
118. Besnard S, Bakouche J, Lemaigre-Dubreuil Y, Mariani J, Tedgui A, Henrion D. Smooth muscle dysfunction in resistance arteries of the staggerer mouse, a mutant of the nuclear receptor RORalpha. *Circ Res* 2002;90(7):820–825.
119. Vecchione C,Fratta L, Rizzoni D, Notte A, Poulet R, Porteri E, Frati G, Guelfi D, Trimarco V, Mulvany MJ, Agabiti-Rosei E, Trimarco B, Cotecchia S, Lembo G. Cardiovascular influences of alpha1b-adrenergic receptor defect in mice. *Circulation* 2002;105(14): 1700–1707.
120. Gross V, Luft FC. Adapting renal and cardiovascular physiology to the genetically hypertensive mouse. *Semin Nephrol* 2002;22 (2):172–179.
121. Holschneider DP, Scremin OU, Roos KP, Chialvo DR, Chen K, Shih JC. Increased baroreceptor response in mice deficient in monoamine oxidase A and B. *Am J Physiol Heart Circ Physiol* 2002;282(3):H964–972.
122. Edouga D, Hugueny B, Gasser B, Bussieres L, Laborde K. Recovery after relief of fetal urinary obstruction: morphological, functional and molecular aspects. *Am J Physiol Renal Physiol* 2001;281(1): F26–37.
123. Kitagawa H, Pringle KC, Zuccollo J, Koike J, Nakada K, Ikoma M, Seki Y. Glomerular size in renal dysplasia secondary to obstructive uropathy: a further exploration of the fetal lamb model. *J Pediatr Surg* 2000;35(11):1651–1615.
124. Kitagawa H, Pringle KC, Zucollo J, Koike J, Nakada K, Moriya H, Seki Y. Early fetal obstructive uropathy produces Potter's syndrome in the lamb. *J Pediatr Surg* 2000;35(11):1549–1553.
125. Smith LM, Ervin MG, Wada N, Ikegami M, Polk DH, Jobe AH. Antenatal glucocorticoids alter postnatal preterm lamb renal and cardiovascular responses to intravascular volume expansion. *Pediatr Res* 2000;47(5):622–627.
126. Kitagawa H, Pringle KC, Zuccolo J, Stone P, Nakada K, Kawaguchi F, Nakada M, Wakisaka M, Furuta S, Koike J, Seki Y. The pathogenesis of dysplastic kidney in a urinary tract obstruction in the female fetal lamb. *J Pediatr Surg* 1999;34(11):1678–1683.
127. Wang J, Rose JC. Developmental changes in renal renin mRNA half-life and responses to stimulation in fetal lambs. *Am J Physiol* 1999;277(4 Pt 2):R1130–1135.
128. Gimonet V, Bussieres L, Medjebeur AA, Gasser B, Lelongt B, Laborde K. Nephrogenesis and angiotensin II receptor subtypes gene expression in the fetal lamb. *Am J Physiol* 1998;274(6 Pt 2): F1062–1069.
129. Nguyen HT, Kogan BA. Renal hemodynamic changes after complete and partial unilateral ureteral obstruction in the fetal lamb. *J Urol* 1998;160(3 Pt 2):1063–1069.
130. Wang J, Perez FM, Rose JC. Developmental changes in renin-containing cells from the ovine fetal kidney. *J Soc Gynecol Investig* 1997;4(4):191–196.
131. Berry LM, Polk DH, Ikegami M, Jobe AH, Padbury JF, Ervin MG. Preterm newborn lamb renal and cardiovascular responses after fetal or maternal antenatal betamethasone. *Am J Physiol* 1997;272 (6 Pt 2):R1972–1979.
132. Matsell DG, Bennett T, Bocking AD. Characterization of fetal ovine renal dysplasia after mid-gestation ureteral obstruction. *Clin Invest Med* 1996;19(6):444–452.
133. Peters CA, Gaertner RC, Carr MC, Mandell J. Fetal compensatory renal growth due to unilateral ureteral obstruction. *J Urol* 1993;150 (2 Pt 2):597–600.
134. Peters CA, Docimo SG, Luetic T, Reid LM, Retik AB, Mandell J. Effect of in utero vesicostomy on pulmonary hypoplasia in the fetal lamb with bladder outlet obstruction and oligohydramnios: a morphometric analysis. *J Urol* 1991;146(4):1178–1183.
135. Rosines E, Sampogna RV, Johkura K, Vaughn DA, Choi Y, Sakurai H, Shah MM, Nigam SK. Staged in vitro reconstitution and implantation of engineered rat kidney tissue. *Proc Natl Acad Sci USA* 2007;104(52):20938–20943.
136. Fissell WH. Developments towards an artificial kidney. *Expert Rev Med Devices* 2006;3(2):155–165.
137. Hammerman MR. Tissue engineering the kidney. *Kidney Int* 2003;63(4):1195–1204.
138. Chugh S, Yuan H, Topham PS, Haydar SA, Mittal V, Taylor GA, Kalluri R, Salant DJ. Aminopeptidase A: a nephritogenic target antigen of nephrotoxic serum. *Kidney Int* 2001;59(2): 601–613.
139. Cook HT, Khan SB, Allen A, Bhangal G, Smith J, Lobb RR, Pusey CD. Treatment with an antibody to VLA-1 integrin reduces glomerular and tubulointerstitial scarring in a rat model of crescentic glomerulonephritis. *Am J Pathol* 2002;161 (4):1265–1272.
140. Hiromura K, Haseley LA, Zhang P, Monkawa T, Durvasula R, Petermann AT, Alpers CE, Mundel P, Shankland SJ. Podocyte expression of the CDK-inhibitor p57 during development and disease. *Kidney Int* 2001;60(6):2235–2246.
141. Lin F, Emancipator SN, Salant DJ, Medof ME. Decay-accelerating factor confers protection against complement-mediated podocyte injury in acute nephrotoxic nephritis. *Lab Invest* 2002;82 (5):563–569.
142. Topham PS, Csizmadia V, Soler D, Hines D, Gerard CJ, Salant DJ, Hancock WW. Lack of chemokine receptor CCR1 enhances Th1 responses and glomerular injury during nephrotoxic nephritis. *J Clin Invest* 1999;104(11):1549–1557.
143. Xu Y, Berrou J, Chen X, Fouqueray B, Callard P, Sraer JD, Rondeau E. Induction of urokinase receptor expression in nephrotoxic nephritis. *Exp Nephrol* 2001;9(6):397–404.
144. Yanagita M, Ishimoto Y, Arai H, Nagai K, Ito T, Nakano T, Salant DJ, Fukatsu A, Doi T, Kita T. Essential role of Gas6 for glomerular injury in nephrotoxic nephritis. *J Clin Invest* 2002;110 (2):239–246.
145. Carmago S, Shah SV, Walker PD. Meprin, a brush-border enzyme, plays an important role in hypoxic/ischemic acute renal tubular injury in rats. *Kidney Int* 2002;61(3):959–966.

146. Chatterjee PK, Brown PA, Cuzzocrea S, Zacharowski K, Stewart KN, Mota-Filipe H, McDonald MC, Thiemermann C. Calpain inhibitor-1 reduces renal ischemia/reperfusion injury in the rat. *Kidney Int* 2001;59(6):2073–2083.
147. Fernandez M, Medina A, Santos F, Carbajo E, Rodriguez J, Alvarez J, Cobo A. Exacerbated inflammatory response induced by insulin-like growth factor I treatment in rats with ischemic acute renal failure. *J Am Soc Nephrol* 2001;12(9):1900–1907.
148. Gimelreich D, Popovtzer MM, Wald H, Pizov G, Berlatzky Y, Rubinger D. Regulation of ROMK and channel-inducing factor (CHIF) in acute renal failure due to ischemic reperfusion injury. *Kidney Int* 2001;59(5):1812–1820.
149. Gretz N. The development of hypertension in the remnant kidney model after either pole resection or partial infarction of the kidney. *J Am Soc Nephrol* 1995;5(10):1839–1840.
150. Jia ZQ, Worthington AE, Hill RP, Hunt JW. The effects of artery occlusion on temperature homogeneity during hyperthermia in rabbit kidneys in vivo. *Int J Hyperthermia* 1997;13(1):21–37.
151. Kakoki M, Hirata Y, Hayakawa H, Suzuki E, Nagata D, Nishimatsu H, Kimura K, Goto A, Omata M. Effects of vasodilatory antihypertensive agents on endothelial dysfunction in rats with ischemic acute renal failure. *Hypertens Res* 2000;23(5):527–533.
152. Knoll T, Schult S, Birck R, Braun C, Michel MS, Bross S, Juenemann KP, Kirchengast M, Rohmeiss P. Therapeutic administration of an endothelin-A receptor antagonist after acute ischemic renal failure dose-dependently improves recovery of renal function. *J Cardiovasc Pharmacol* 2001;37(4):483–488.
153. Kren S, Hostetter TH. The course of the remnant kidney model in mice. *Kidney Int* 1999;56(1):333–337.
154. Kwon O, Phillips CL, Molitoris BA. Ischemia induces alterations in actin filaments in renal vascular smooth muscle cells. *Am J Physiol Renal Physiol* 2002;282(6):F1012–1019.
155. Lieberthal W, Fuhro R, Andry CC, Rennke H, Abernathy VE, Koh JS, Valeri R, Levine JS. Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol Renal Physiol* 2001;281(4):F693–706.
156. Lloberas N, Torras J, Herrero-Fresneda I, Cruzado JM, Riera M, Hurtado I, Grinyo JM. Postischemic renal oxidative stress induces inflammatory response through PAF and oxidized phospholipids. *Prevention by antioxidant treatment. Faseb J* 2002;16(8):908–910.
157. Megyesi J, Andrade L, Vieira JM Jr., Safirstein RL, Price PM. Positive effect of the induction of p21WAF1/CIP1 on the course of ischemic acute renal failure. *Kidney Int* 2001;60(6):2164–2172.
158. Meldrum KK, Hile K, Meldrum DR, Crone JA, Gearhart JP, Burnett AL. Simulated ischemia induces renal tubular cell apoptosis through a nuclear factor-kappaB dependent mechanism. *J Urol* 2002;168(1):248–252.
159. Modolo NS, Castiglia YM, Ganem EM, Braz JR, Vianna PT, Vane LA. Acute renal ischemia model in dogs: effects of metoprolol. *Ren Fail* 2001;23(1):1–10.
160. Mister M, Noris M, Szymczuk J, Azzollini N, Aiello S, Abbate M, Trochimowicz L, Gagliardini E, Arduini A, Perico N, Remuzzi G. Propionyl-L-carnitine prevents renal function deterioration due to ischemia/reperfusion. *Kidney Int* 2002;61(3):1064–1078.
161. Okusa MD. The inflammatory cascade in acute ischemic renal failure. *Nephron* 2002;90(2):133–138.
162. Power JM, Tonkin AM. Large animal models of heart failure. *Aust N Z J Med* 1999;29(3):395–402.
163. Textor SC. Pathophysiology of renal failure in renovascular disease. *Am J Kidney Dis* 1994;24(4):642–651.
164. Vaneerdeweg W, Buyssens N, De Winne T, Sebrechts M, Babloyan A, Arakelian S, De Broe ME. A standardized surgical technique to obtain a stable and reproducible chronic renal failure model in dogs. *Eur Surg Res* 1992;24(5):273–282.
165. Yoshida T, Tang SS, Hsiao LL, Jensen RV, Ingelfinger JR, Gullans SR. Global analysis of gene expression in renal ischemia-reperfusion in the mouse. *Biochem Biophys Res Commun* 2002;291(4):787–794.
166. Chevalier RL. Chronic partial ureteral obstruction and the developing kidney. *Pediatr Radiol* 2008;38 Suppl 1:S35–40.
167. Ma FY, Tesch GH, Flavell RA, Davis RJ, Nikolic-Paterson DJ. MKK3-p38 signaling promotes apoptosis and the early inflammatory response in the obstructed mouse kidney. *Am J Physiol Renal Physiol* 2007;293(5):F1556–1563.
168. Chevalier RL. Pathogenesis of renal injury in obstructive uropathy. *Curr Opin Pediatr* 2006;18(2):153–160.
169. Foster MH. Relevance of systemic lupus erythematosus nephritis animal models to human disease. *Semin Nephrol* 1999;19(1):12–24.
170. Morel L and Wakeland EK. Susceptibility to lupus nephritis in the NZB/W model system. *Curr Opin Immunol* 1998;10(6):718–725.
171. Walport MJ, Davies KA, Botto M. C1q and systemic lupus erythematosus. *Immunobiology* 1998;199(2):265–285.
172. Gavalchin J, Staines NA. T and B cell recognition of idiotypes of anti-DNA autoantibodies. *Lupus* 1997;6(3):337–343.
173. Isenberg DA, Ravirajan CT, Rahman A, Kalsi J. The role of antibodies to DNA in systemic lupus erythematosus–a review and introduction to an international workshop on DNA antibodies held in London, May 1996. *Lupus* 1997;6(3):290–304.
174. Pickering MC, Cook HT, Warren J, Bygrave AE, Moss J, Walport MJ, Botto M. Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat Genet* 2002;31(4):424–428.
175. Salvador JM, Hollander MC, Nguyen AT, Kopp JB, Barisoni L, Moore JK, Ashwell JD, Fornace AJ, Jr. Mice lacking the p53-effector gene Gadd45a develop a lupus-like syndrome. *Immunity* 2002;16(4):499–508.
176. Tabata N, Miyazawa M, Fujisawa R, Takei YA, Abe H, Hashimoto K. Establishment of monoclonal anti-retroviral gp70 autoantibodies from MRL/lpr lupus mice and induction of glomerular gp70 deposition and pathology by transfer into non-autoimmune mice. *J Virol* 2000;74(9):4116–4126.
177. Cruse JM, Lewis RE, Dilioglou S. Fate of immune complexes, glomerulonephritis, and cell-mediated vasculitis in lupus-prone MRL/Mp lpr/lpr mice. *Exp Mol Pathol* 2000;69(3):211–222.
178. Ophascharoensuk V, Fero ML, Hughes J, Roberts JM, Shankland SJ. The cyclin-dependent kinase inhibitor p27Kip1 safeguards against inflammatory injury. *Nat Med* 1998;4(5):575–580.
179. Cattell V, Cook HT, Ebrahim H, Waddington SN, Wei XQ, Assmann KJ, Liew FY. Anti-GBM glomerulonephritis in mice lacking nitric oxide synthase type 2. *Kidney Int* 1998;53(4):932–936.
180. Quigg RJ, Lim A, Haas M, Alexander JJ, He C, Carroll MC. Immune complex glomerulonephritis in C4- and C3-deficient mice. *Kidney Int* 1998;53(2):320–330.
181. Tang T, Rosenkranz A, Assmann KJ, Goodman MJ, Gutierrez-Ramos JC, Carroll MC, Cotran RS, Mayadas TN. A role for Mac-1 (CDIIb/CD18) in immune complex-stimulated neutrophil function in vivo: Mac-1 deficiency abrogates sustained Fcgamma receptor-dependent neutrophil adhesion and complement-dependent proteinuria in acute glomerulonephritis. *J Exp Med* 1997;186(11):1853–1863.

182. Ito MR, Terasaki S, Itoh J, Katoh H, Yonehara S, Nose M. Rheumatic diseases in an MRL strain of mice with a deficit in the functional Fas ligand. *Arthritis Rheum* 1997;40(6):1054–1063.
183. Haas C, Ryffel B, Le Hir M. IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/Ipr mice. *J Immunol* 1997;158(11):5484–5491.
184. Hibbs ML, Tarlinton DM, Armes J, Grail D, Hodgson G, Maglitto R, Stacker SA, Dunn AR. Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. *Cell* 1995;83(2):301–311.
185. Cyster JG. Lymphoid organ development and cell migration. *Immunol Rev* 2003;195:5–14.
186. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. *Nature* 2001;410(6824):101–105.
187. Gudmundsdottir H, Turka LA. T cell costimulatory blockade: new therapies for transplant rejection. *J Am Soc Nephrol* 1999;10(6):1356–1365.
188. Dong VM, Womer KL, Sayegh MH. Transplantation tolerance: the concept and its applicability. *Pediatr Transplant* 1999;3(3):181–192.
189. Bromberg JS, Murphy B. Routes to allograft survival. *J Clin Invest* 2001;107(7):797–798.
190. Light J, Salomon DR, Diethelm AG, Alexander JW, Hunsicker L, Thistlethwaite R, Reinsmoen N, Stablein DM. Bone marrow transfusions in cadaver renal allografts: pilot trials with concurrent controls. *Clin Transplant* 2002;16(5):317–324.
191. Knechtle SJ, Hamawy MM, Hu H, Fechner JH, Jr, Cho CS. Tolerance and near-tolerance strategies in monkeys and their application to human renal transplantation. *Immunol Rev* 2001;183:205–213.
192. Inverardi L, Ricordi C. Tolerance and pancreatic islet transplantation. *Philos Trans R Soc Lond B Biol Sci* 2001;356(1409):759–765.
193. Field EH, Strober S. Tolerance, mixed chimerism and protection against graft-versus-host disease after total lymphoid irradiation. *Philos Trans R Soc Lond B Biol Sci* 2001;356(1409):89–95.
194. Decker CJ, Heiser AD, Chaturvedi PR, Faust TJ, Ku G, Moseley S, Nimmesgern E. The novel IMPDH inhibitor VX-497 prolongs skin graft survival and improves graft versus host disease in mice. *Drugs Exp Clin Res* 2001;27(3):89–95.
195. Yoshimura R, Chargui J, Aitouche A, Veyron P, Touraine JL. Induction of hyperacute rejection of skin allografts by CD8 + lymphocytes. *Transplantation* 2000;69(7):1452–1457.
196. Gardner CR. The pharmacology of immunosuppressant drugs in skin transplant rejection in mice and other rodents. *Gen Pharmacol* 1995;26(2):245–271.
197. Tepper MA, Linsley PS, Tritschler D, Esselstyn JM. Tolerance induction by soluble CTLA4 in a mouse skin transplant model. *Transplant Proc* 1994;26(6):3151–3154.
198. Sho M, Sandner SE, Najafian N, Salama AD, Dong V, Yamada A, Kishimoto K, Harada H, Schmitt I, Sayegh MH. New insights into the interactions between T-cell costimulatory blockade and conventional immunosuppressive drugs. *Ann Surg* 2002;236(5):667–675.
199. Rolls HK, Kishimoto K, Dong VM, Illigens BM, Sho M, Sayegh MH, Benichou G, Fedoseyeva EV. T-cell response to cardiac myosin persists in the absence of an alloimmune response in recipients with chronic cardiac allograft rejection. *Transplantation* 2002;74(7):1053–1057.
200. Zhai Y, Meng L, Gao F, Busuttil RW, Kupiec-Weglinski JW. Allograft rejection by primed/memory CD8 + T cells is CD154 blockade resistant: therapeutic implications for sensitized transplant recipients. *J Immunol* 2002;169(8):4667–4673.
201. Fedoseyeva EV, Kishimoto K, Rolls HK, Illigens BM, Dong VM, Valujskikh A, Heeger PS, Sayegh MH, Benichou G. Modulation of tissue-specific immune response to cardiac myosin can prolong survival of allogeneic heart transplants. *J Immunol* 2002;169(3):1168–1174.
202. Coates PT, Krishnan R, Kireta S, Johnston J, Russ GR. Human myeloid dendritic cells transduced with an adenoviral interleukin-10 gene construct inhibit human skin graft rejection in humanized NOD-scid chimeric mice. *Gene Ther* 2001;8(16):1224–1233.
203. Fahy O, Porte H, Senechal S, Vorng H, McEuen AR, Buckley MG, Walls AF, Wallaert B, Tonnel AB, Tsicopoulos A. Chemokine-induced cutaneous inflammatory cell infiltration in a model of Hu-PBMC-SCID mice grafted with human skin. *Am J Pathol* 2001;158(3):1053–1063.
204. Moulton KS, Melder RJ, Dharnidharka VR, Hardin-Young J, Jain RK, Briscoe DM. Angiogenesis in the huPBL-SCID model of human transplant rejection. *Transplantation* 1999;67(12):1626–1631.
205. Briscoe DM, Dharnidharka VR, Isaacs C, Downing G, Prosky S, Shaw P, Parenteau NL, Hardin-Young J. The allogeneic response to cultured human skin equivalent in the hu-PBL-SCID mouse model of skin rejection. *Transplantation* 1999;67(12):1590–1599.
206. Hammerman MR. Xenotransplantation of renal primordia. *Curr Opin Nephrol Hypertens* 2002;11(1):11–16.
207. Palmer DB, Lechler R. Can the thymus be a useful tool to induce specific tolerance to xenoantigens? *Transplantation* 1999;68(11):1628–1630.