

# Phenotyping of Genetically Altered Mice

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## 1. INTRODUCTION

Genetically altered mice provide superb models of human physiology and disease. They allow us to evaluate the effects of single altered genes in the context the whole organism and provide tremendous insight into gene function. However, they can provide research results that are frequently unexpected, confusing or simply uninformative. The comparative pathologist is required to assess phenotypic impact of single gene alterations on complex molecular pathways. The effects of genetic background and the variability inherent in the gene construct used to create the animals frequently confound this assessment. Finally, findings must be integrated with published information to draw conclusions and design new experiments.

The aim of each phenotyping project is unique, however several common features can usually be identified. In most phenotyping studies, the intention is to not only identify the nature of the lesions, but also to assess how such lesions relate to deviation of normal gene expression and cellular physiology. This review will delineate the approach taken during a typical phenotypic assessment, with particular emphasis on evaluation of embryonic and neurologic phenotypes.

When initiating a phenotypic examination, it is important to collect as much information about the experiment as possible. These include: a) the aim and design of the experiment b) the known physiology of the target gene and the methods used to manipulate its expression and c) potential sources of phenotypic variability. The latter factor can easily obscure subtle phenotypes. A critical assessment of these factors is necessary to accurately ascribe altered function of the gene target to the observed phenotypic change. On a more practical level, these factors determine the number and age of animals that must be examined, as well as which tissues should be included in the screening panel. Sources of phenotypic variability include the following:

**Method of genetic manipulation.** Understanding the technology used in the experiment is necessary in order to identify potential factors which may confound the phenotype. Detailed descriptions of methodologies used to create genetically altered animals can be found in Williams and Wagner 2000. The following discussion will address only pitfalls associated with the more commonly used methods to generate transgenic and knockout mice.

There are two basic approaches to manipulate the mouse genome. The first method is used to create **transgenic** animals and employs random chromosomal integration of foreign DNA following injection into fertilized oocytes. The resulting offspring are screened to identify those animals in which stable chromosomal integration of the foreign DNA has occurred. In addition to the target gene, the transgenic construct contains a transcriptional regulatory region which directs both expression level and tissue specificity of the inserted gene. Depending on the aim of the experiment, the target protein may be overexpressed (excessive amounts of normal protein expressed in tissues which normally express it), or ectopically expressed (a normal protein is expressed in tissues which do not normally express it). Alternatively, the transgene may be modified to create a "gain of function" mutant (where the protein is constitutively expressed) or a "loss of function" mutant (where the protein interacts with its partners in a dominant negative fashion). The nature of the transgenic manipulation will determine the extent to which individual tissues are examined.

Because of the random nature of transgene insertion after pronuclear injection, each resultant founder contains the transgene at a different site in the genome (Clark et al., 1994). This **position effect** can profoundly affect the expression of both the transgene and endogenous genes whose regulatory elements may be disrupted by the insertion event. Several factors may influence the resultant phenotype. The foreign DNA usually integrates as linear arrays, resulting in variable levels of gene dosage. The site of chromosomal integration may affect the regulatory function of the transcriptional element contained within the construct. These factors result in variable expression levels of the transgene in different founder lines. In addition, random integration of the transgene may disrupt endogenous genes (insertional mutagenesis) thus further confounding phenotype. Consequently, it is essential that lines from several (at least two) different founders be examined before a conclusion relating a specific phenotype to transgene expression is made (Sigmund C, 2000; Williams and Wagner 2000). To assess dose-response relationships between transgene expression and phenotype, it is also important to assess lines of mice which express the transgene at different levels.

The uncertainties of random integration may be circumvented by the more challenging technology used to create **knockout** mice. Using homologous recombination, the coding region of a specific endogenous gene can be interrupted to eliminate gene expression ("knockout") or replaced with a modified variant of the gene ("knock-in"). The foreign DNA is inserted into cultured ES cells, followed by identification of clones that have the correct mutation and then injection of these clones into mouse blastocysts. If chimeric mice have integrated the foreign DNA into their germline, they can pass it along to their progeny to establish a colony of genetically altered animals. Although gene expression may be more precisely controlled with this method, it is possible to destroy transcriptional control elements controlling expression of a neighboring gene, thus creating varying phenotypes (Olson et al., 1996).

More sophisticated methods of genetic manipulation are accompanied by their own particular pitfalls. These methods include Cre-Lox technology to create conditional mutants and drug-regulated transgene expression.

**Genetic background.** Most commonly used ES cell lines are derived from several 129 substrains (Simpson et al., 1997). Chimeric animals are typically bred to C57BL/6 mice, producing genetically similar F1 animals sharing similar chromosomal complements of 129 and C57BL/6 strains. However, when these animals are crossed to generate F2 animals homozygous for the target locus, the offspring have a random mix of 129 and C57BL/6 chromosomal DNA. Consequently, phenotypic variability may be caused by alleles which are not linked to the target locus. The increasing use of double/triple knockout combinations, inducible transgenes and cell-specific knockouts provide additional opportunities for creating a genetic background so mixed that results cannot be replicated by other investigators. Ideally, the genetic background of control and experimental animals should be identical, with exception of the target locus. In cases where background effects are likely to be important, the target locus is best propagated in **congenic** strains by successive backcrossing to one inbred strain. After breeding parental strains, F1 progeny are bred back to one parental strain (usually C57BL/6). F2 progeny from this mating are then similarly bred back to the parental strain until, after 6 backcross breedings (a process which generally takes two years), the resultant offspring are 99% similar to the chosen strain, with the exception of the region surrounding the target locus. This strategy also provides the opportunity to place the target locus on a number of genetic backgrounds to assess the effects of strain-specific modifier loci (Sigmund C, 2000). However, circumstances frequently dictate that phenotype of genetically altered animals be evaluated long before congenic strains can be generated. In these cases, wild-type and heterozygous littermates from the same breedings are used as controls. To ensure that the range of phenotypes possible due to genetic recombination is assessed, large numbers of animals should be examined.

**Compensation.** When a gene of interest is disrupted, a compensatory process may take over the function of the missing gene and mask its phenotypic outcome (Susulic et al., 1995; Cummings et al., 1996). Such compensation is typically identified by the altered or increased expression of related genes in the presence of a relatively normal phenotype.

**Epigenetic phenomena.** Environmental phenomena such as stress and food composition can have substantial effects on phenotype. In particular, behavioral phenotypes, or those phenotypes such as obesity which are affected by behavior and feeding can be particularly affected (Crabbe et al., 1999; Tordoff et al., 1999).

**Infectious disease status.** If animals have been produced at the research facility, information regarding the health status of the room in which they live will be available. In general, those phenotypes which affect the immune system are most likely to suffer potential confounding effects of a prevalent but subclinical infectious disease. Not infrequently, Helicobacteriosis will present as clinical disease (rectal prolapse), particularly in animals prone to inflammatory bowel disease (Chin et al., 2000) as the result of ablation of components of their immune system.

**Strain-specific or age-related background pathology.** Strain-specific anatomy and pathology is described and referenced in several excellent texts (Maronpot et al., 1999; Hof et al., 2000; Ward et al., 2000; Brayton et al., 2001). In addition, online resources such as the Mouse Phenome Database, the database of Inbred Strain Characteristics, and the Mouse Tumor Biology Database provide searchable databases of strain-specific anatomy and pathology (see Table 3). The latter is complemented by a recent text on murine tumor classification (Mohr, 2001).

## **2. THE INITIAL ANATOMIC PHENOTYPING SCREEN**

**Selection of mutant and control animals.** Two issues bear special consideration when selecting animals for morphologic evaluation - the age at which animals are evaluated and their genetic background. The window during which the phenotype is expressed will determine the optimal age for comprehensive examination. One particular possibility which should always be assessed is whether prenatal death of mutant animals is occurring. Disproportionate reduction in the number of homozygous mutant animals compared to numbers of heterozygous and wild-type siblings is usually the first indication of this problem. Identifying the time of fetal death requires euthanasia of pregnant dams at successive stages of pregnancy to determine the time at which embryos are lost. Good reviews detailing the evaluation of embryonic death and perinatal mortality can be found in Brayton et al, 2001 and Ward et al, 2000. If clinical abnormalities are present in postnatal animals, the investigator is in the best position to detail the onset and progression of such signs. Numerous protocols for the ante-mortem physiologic assessment of mutant mice exist. These are succinctly reviewed in Rao and Verkman 2000. For progressive conditions, animals in early, mid and late stages of the condition should be chosen for histologic analysis. Not uncommonly, no clinical abnormalities are noted at all. In this case, baseline phenotyping can be performed in young (8-12 weeks) and older (12-15 months) adult animals.

The effects of genetic background on phenotype are well documented, and can be substantive enough to obscure subtle phenotypes originating from the induced genetic alteration. Ideal candidates for phenotypic evaluation would be backcrossed  $-/-$ ,  $+/-$  and  $+/+$  littermates from at least the 5th generation. These animals would be approximately 95% genetically similar, and would differ only in the region surrounding the target gene. In practice, the constraints of time, cost and potential loss of the phenotype from multiple backcrosses result in presentation of animals of more mixed background for examination. This does not pose particular problems if the phenotype attributable to the target gene is an obvious one. However, the more subtle the phenotype, the greater the number of animals that are needed to extract a significant result from the experiment. At a minimum, two age matched, sex-matched individuals of each genotype (at each time point, and in the case of transgenic animals, from each founder line) are needed to obtain baseline histologic data

**Gross necropsy and fixation.** The pathologic evaluation of mutant mice relies on methods common to veterinary diagnostic pathology. The necessary technology and procedures used to perform basic assessment of mutant mice have been comprehensively described in several reviews and books (Ward et al., 2000; Brayton et al., 2001; Sundberg 1994; 1996; 2000). Specific organ systems have been reviewed by Car and Eng (2001; hematology), and Sundberg (2000; skin).

Following euthanasia, blood is collected by cardiac puncture for clinical pathology. In the initial screen, this comprises a complete blood count and clinical chemistry. Bone marrow smears should be taken at this time, well as a blood smear. Urine may be collected prior to euthanasia (mice will often urinate on a piece of parafilm if handled) or by cystocentesis after euthanasia. Normal murine clinical pathology values may differ from laboratory to laboratory, and should be established by submitting blood from several normal mice prior to evaluating actual cases. A list of markers used to identify individual cellular components of blood may be found in Car and Eng, 2001.

After exsanguination, the mouse is weighed. It is placed in dorsal recumbancy and a standard necropsy performed. For an on-line demonstration of a mouse necropsy see [http://www.eulep.org/Necropsy\\_of\\_the\\_Mouse/](http://www.eulep.org/Necropsy_of_the_Mouse/)) Organs are removed and weighed (usually limited to major organs), and organ weights expressed as percentage of total body weight. Grossly evident pathology is noted and photographed (with ruler and identification label) at this stage. This is also the point at which tissues may be collected for molecular techniques.

The entire mouse may be fixed in 10% formalin, 4% paraformaldehyde, Bouin's solution or Feketes acid alcohol. 10% formalin is adequate for initial screening, and Bouin's solution and Feketes acid alcohol are usually used for the nervous system. All except Bouin's solution require decalcification of bony tissues prior to embedding. Bouin's solution will decalcify as it fixes, but care must be taken not to overfix the tissue (in the case of embryos and neonates), and the fixative must be rinsed with water until it runs clear prior to placing tissues in 70% ethanol for storage. Recipes for Feketes acid alcohol and Bouin's solution are given below.

**Feketes acid alcohol.**

700ml/L 100% ethanol  
32 ml/L 37% formalin  
40ml/L glacial acetic acid  
dd water to 1L

**Bouins solution**

160 ml 37% formalin  
80 ml glacial acetic acid  
1700 ml saturated picric acid

Fixation by intracardiac perfusion is recommended for tissues which autolyse rapidly, such as nervous tissue or endocrine tissue. A surgical depth of anesthesia is attained (absence of withdrawel reflex when the foot is firmly pinched with forceps), the mouse is pinned in dorsal recumbancy. The chest is opened, and the right atrium removed with scissors. A 21ga butterfly needle is placed in the left ventricle, and 5-20 ml of saline flushed in over the course of about a minute. Thereafter, 30-50 ml of fixative is flushed in

until the body becomes stiff. The skull is then opened, and the abdomen opened along the midline before the mouse is immersed in fixative for another 7-10 days.

**Histologic evaluation.** A list of tissues typically examined during the initial screen is given below. Often, this may be adequate. However, in many cases, specific organs systems are suspected to contain more subtle lesions. In these cases, detailed evaluation of individual organs systems can be done. These most commonly include serial sectioning (usually embryos or organs with complex 3D structure such as the heart and brain), or immunohistochemistry using paraffin-embedded or frozen sections. Additional animals at different ages may also be required.

#### Tissues evaluated during initial screen.

- a. Head – nasal passages, teeth, skin, vomeronasal organ, eyes, inner, middle and outer ear, tongue, salivary glands, brain, pituitary gland, bone.
- b. Heart (longitudinal section), lung (whole lung), trachea and thyroid glands.
- c. Liver, gall bladder, adrenal glands and kidneys (in transverse and longitudinal section).
- d. Stomach, duodenum, jejunum, pancreas, mesenteric lymph nodes.
- e. Ileum, cecum and colon, rectum.
- f. Urinary bladder, testes, accessory sex glands, preputial gland (in males) or uterus, ovaries, clitoral gland and mammary gland (in females).
- g. Cervical lymphoglandular complex (cervical lymph node, submandibular, submaxillary and parotid lymph nodes), diaphragm.
- h. Hind and forelimbs and sternum (skeletal muscle, bone marrow, peripheral nerve, skin, joints)

### **3. EMBRYOS**

**Collection of embryos at specific developmental stages.** Matings between fertile males and spontaneously cycling females are usually set up in the late afternoon or early evening. Females in proestrus can be selected by vaginal inspection (Champlin et al., 1973). Approximately half of the females selected this way will mate that night. Consequently, a relatively large number of matings need to be set up in order to obtain the required number of timed pregnant females. Alternatively, females can be superovulated using intraperitoneal pregnant mares serum gonadotropin (PMSG, typical dose 5IU) followed 48 hours later by human chorionic gonadotropin (HCG, typical dose 5IU). Ovulation occurs approximately 12 hours later. Depending on the dose administered, ranging from 2.5 IU (physiologic) to 10 IU (high), large numbers of embryos may implant and result in artifactual changes from overcrowding (Kaufmann MH, 2000).

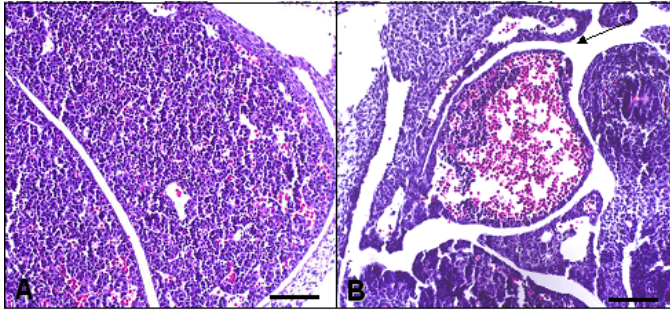
Observation of a vaginal plug is required to accurately determine the developmental stage of embryos. In mice kept in a standard 12 hour light:dark cycle, it is assumed that mating occurs at the mid-dark point, at approximately 2 am. If a vaginal plug is identified the

next morning, embryos will be assumed to be E 0.5 (embryonic day) or 0.5 dpc (days post coitum) old (Copp and Cockcroft 1990; Hogan et al., 1994). Implantation usually occurs at E4.5 and the duration of pregnancy is 19.5-21 days. Before implantation, embryos may be retrieved by flushing the oviduct and uterus with phosphate buffered saline. Between E4.5 and E8.5, it is best to isolate the embryo within its intact decidual swelling to avoid damaging it. After E8.5, the embryo can be dissected from the uterus and its yolk sac. It can be retained within the amnion, but considerable care should be taken to avoid damage.

**Fixation, embedding and orientation.** Embryos may be fixed in Bouin's solution, 10% formalin or 4% paraformaldehyde. Particularly with Bouin's solution, the tissue will become brittle if placed in fixative for too long. Embryos with a crown-rump length of 2mm require only 1 hour in fixative, while those with a crown-rump length of approximately 15 mm can be placed in fixative for up to 24 hours. After removal from fixative, embryos may be placed in 70% ethanol for long-term storage at room temperature. Before embedding, the embryos are dehydrated through graded stages of alcohol, prior to being placed in a 1:1 mixture of 100% ethanol: benzene (see Kaufmann 1990 for detailed procedures). The addition of a few drops of eosin at the 90% ethanol stage will stain the embryo pink and facilitate its visualization during embedding. Embryos older than E8.5 can be relatively easily oriented, as the head and tail can be easily visualized, and they tend to fall on their sides in the wax block. Younger embryos within their decidual swellings can be sectioned in the transverse plane by using the decidual swelling to orient the embryo. Attempting to section in the longitudinal plane often produces sections of intermediate obliquity. A large number of specimens may be required to obtain useful sections of specimens under E8.5. For most purposes, embryos are examined by collecting serial sections cut at 5-8  $\mu$ m in the sagittal and transverse planes. Transverse sections are generally done through the majority of the embryo, and provide the most morphologic information. Sagittal sections are usually restricted to a few sections obtained on either side of the midline, as these become less informative the further from the median plane they are. Coronal sections are usually reserved for examining the cephalic region of E14.5-E15.5 embryos.

**Histologic interpretation and staging.** The most commonly used staging system is that of Theiler (Theiler 1972;1989). This system has been adopted by recent standard texts on mouse embryology (Kaufman 1994; Kaufman and Bard 1999). A table correlating the Theiler system with embryonic age, size and morphologic features can be found at <http://genex.hgu.mrc.ac.uk/>. The *Atlas of Mouse Development* (Kaufman, 1994) provides the most comprehensive illustration of each of the Theiler stages. Each Theiler stage, up to about E11.5 (Theiler stage 20) lasts for about 12 hours. As tissues develop so rapidly at these stages, a precise identification of embryo age may be difficult. Ageing of embryos is easier after E12, when each Theiler stage encompasses about 24 hours. Ageing can also be done by examining the sequence of long bone ossification in whole embryos or tissue sections. This method is best used after E15.5 (Patton and Kaufman 1995) when ossification centers are present. The pathologist should be aware of intrinsic variations in normal embryonal development. Within the same litter, developmental maturity can vary by 6-12 hours. In addition, the studies of Theiler and Kaufman have

used F1 hybrids between C57BL X CBA mice, and variations in the developmental velocity of other strains are likely to be present.



**Figure 1:** Hematoxylin and eosin stained sections of wild-type (A) and mutant (B) liver at E12.5. The mutant animal has a very small, blood-filled liver, with poorly formed trabeculae (arrow). Bar = 50 microns.

Hematoxylin and eosin staining is sufficient for initial screening. Further analyses frequently make use of the spectrum of techniques traditionally used in light microscopy e.g. special stains, histochemistry (Kaufman and Schnebelen 1986), immunohistochemistry and *in situ* hybridization (Durrant 1996; Kadkol et al 1999). In these cases, additional embryos which have been fixed and processed according to the needs of the specific protocol are usually required. Evaluation of the embryonal skeleton is best done using alcian blue-alizarin red staining of whole cleared embryos (Patton and Kaufman 1995).

#### 4. NEUROANATOMICAL EVALUATION

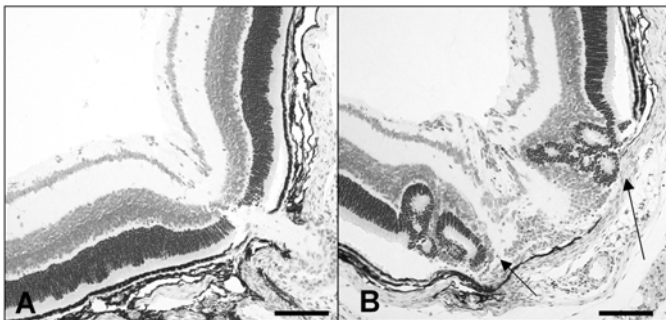
**Clinical evaluation of the live mouse.** Forebrain lesions rarely induce neurologic signs and require behavioral testing to detect. Clinical examination should precede behavioral testing as a host of unrelated factors can cause profound artifactual deficits on behavioral tests. Strain-specific background pathology (e.g. retinal degeneration in C3H and related mice, or callosal defects in BALB/c, 129 and other mice) may affect behavioral test results. C57BL/6 mice tend to display “normal” hyperactive behaviours compared to other strains. Locomotor abnormalities and muscle weakness may also result from abnormalities of the cardiovascular and hematologic systems, or from generalized malaise due to renal or hepatic disease. Clinically apparent neurologic lesions generally result from lesions in the brain stem, cerebellum, vestibular system, spinal cord, nerve or muscle. Table 1 lists tests and clinical signs which may be used to localize lesions to specific regions of the nervous system. Table 2 lists the most common behavioral tests used to assess more subtle motor, sensory or cognitive capacities.

**Tissue collection.** Perfusion (see above) or immersion fixation can be used. The former is desirable particularly if the inner ear is to be examined. Decalcification will occur over a period of about 6-10 days (adult mice) with Bouin’s solution. Other fixatives require 24 - 48 hours of decalcification. As removal of the central nervous system prior to fixation results in significant artifact, we usually fix the central nervous system (CNS) *in situ*, and section the decalcified tissues. This has the advantage of being able to evaluate the CNS in relation to its surrounding structures. In general, paraffin embedded tissue is used to

obtain an overview of the pathology present – additional techniques such as electron microscopy or morphologic techniques requiring cryosections are performed once the anatomic location of a lesion has been established.

**Histologic examination.** Detecting subtle lesions may require serial sectioning, however it is best to try to identify which region of the brain is likely to contain the lesions before progressing to serial sections. Pathologists are generally most familiar with cross (or coronal) sections of the brain – the nomenclature used by Paxinos and Franklin (2001) is referred to most often when identifying the localization of the section. Using this nomenclature, Bregma positions refer to the level at which the coronal section has been taken, with Bregma 0 mm representing the baseline reference value (located just caudal to the eyes). Positive Bregma values correspond to regions rostral to Bregma 0 mm, and negative values to locations caudal to Bregma 0 mm. Initial examination of the brain can be done using 6-8 sections representative of the following key regions:

- a. Bregma 3.6 mm: eye and olfactory bulb
- b. Bregma 0mm: internal and external capsules, basal ganglia, sensory and motor cortex
- c. Bregma –1mm to –3.8mm: hippocampus, amygdala, hypothalamus, thalamus, cortex, corpus callosum
- d. Bregma –6.3mm: cerebellum, vestibular nuclei, cerebellar nuclei, inner ear.
- e. Bregma –8mm: caudal cerebellum, medulla.



**Figure 2:** Peripapillary retinal dysplasia in a caspase-3 knockout mouse (B). Neuroretinal folds (arrows) are noted around the optic nerve in the mutant animal, but not in the wild-type control mouse (A). Bar = 50 microns, coronal sections at Bregma 3.6 mm.

Several sections each of the cervical, thoracic and lumbar spine should be taken, as well as a sagittal section of a hind limb to visualize skeletal muscle and peripheral nerve. The inner ear should be examined in three axes - coronal, horizontal and paramedian. Many lesions affecting the hair cells may only be detectable using scanning or transmission electron microscopy. Hematoxylin and eosin staining will allow visualization of most lesions –additional special stains, immunohistochemical stains and histochemical stains can be done. More recently, magnetic resonance imaging has been used to characterize neurodevelopmental phenotypes in live mice (Benveniste and Blackband 2002).

## 5. CARDIOVASCULAR AND SKELETAL PHENOTYPING

*In vivo* cardiac phenotyping in mice is complicated by the small size of the heart and its rapid rate (approximately 500 bpm). Large variations in normal heart rate are reported

(200-700 bpm). In addition, anesthesia results in significant reduction in heart rate, blood pressure and body temperature thus complicating evaluation of the unconscious animal. Conversely, restraint of the conscious animal results in artifactual elevation of heart rate and blood pressure. Despite these challenges, numerous non-invasive technologies such as echocardiography, electrocardiography, tail cuffs and radio telemetry have been developed to evaluate live mice. (Berul CI 2003; Collins et al., 2003; Fitzgerald et al., 2003; Svensen et al., 2003). Subclinical phenotypes may reveal abnormalities following physiologic challenge stimuli – these include pharmacologic, dietary, exercise, and emotional challenges. As with other systems, combining mutant strains to generate double mutant animals may also reveal an underlying phenotype (Bernstein D 2003).

Skeletal phenotyping can be accomplished using an array of in vivo and post mortem techniques. Observation of whole animal characteristics (abnormal skeletal features) can be done directly or with radiography. Alcian blue-alizarin red staining to visualize bone and cartilage is best for embryos. More complicated in vivo imaging techniques such as MRI, computed tomography or DEXA are used to evaluate bone density and quality. A review of advanced imaging techniques in live mice can be found in Hoit BD, 2001. In post-mortem tissue, histologic morphometry plays a crucial role. Finally, mechanical tests of bone strength can be done by evaluating resistance to torsion, compression and bending (Boskey et al., 2003).

## 6. INFORMATION RESOURCES

Following completion of the initial phenotyping panel, the data should be assessed in the light of the experimental design. This requires integration of current knowledge of the cellular process in which the target gene is involved, and comparison with described related mouse phenotypes.

**Information sources on gene and phenotype.** Collective analysis of numerous mutant mouse studies will eventually provide a more comprehensive overview of cellular physiology and pathobiology in the whole organism. As sequencing of the mouse genome nears its completion, mutant mouse studies provide the best opportunity for generating a functional correlate of descriptive genomics (i.e structure of genes, and their associated mRNA and protein sequences). Most veterinary pathologists are trained to categorize disease according to organ system or etiology. Because mutant mice are generated by genetic manipulation, and the resulting pathology frequently affects multiple organ systems, this approach is ill-suited to traditional analysis. Currently, no comprehensive resource exists which correlates structure and function of genes to their cognate cellular pathways and mutant phenotypes. However, extensive data exists for each of these disciplines independently, so it falls to the pathologist and investigator to integrate them. Table 3 provides a recent list of the most comprehensive resources. As few central databases exist, the pathologist must collate published information on similar or related mutants. The Entrez site (<http://www.ncbi.nlm.nih.gov/Entrez/>) hosted by NCBI is most suited to this purpose.

**Gene/protein structure and function.** In order to critically evaluate phenotype, the pathologist should be aware of the gene structure, expression pattern of the gene and protein in the embryo and the adult, and the role in cellular physiology. By the time a genetically altered animal is created, a fair amount of information is available about the gene. On-line resources are listed in Table 4. The Unigene site provides the gene sequence and structure, protein sequence, and associated literature. The site can be searched according to organism, and is useful for comparing mouse and human genes. In addition to search by gene, LocusLink allows searches by organ system or cellular process. The latter searches provide a list of functionally related genes and literature. Knowing when and in which tissue the gene is normally expressed is important to select the correct age animals for examination, interpret the resulting phenotype and select the correct repertoire of tissues for detailed examination. Examination of the images in appropriate research articles usually provides the best description of gene expression. In addition, multiple databases provide comprehensive lists of gene expression by tissue, organism and disease/physiologic state (see Table 4). The WWW Virtual Library of Cell Biology ([http://vlib.org/Science/Cell\\_Biology/index.shtml](http://vlib.org/Science/Cell_Biology/index.shtml)) provides numerous links to sites related to most aspects of cellular function.

**Genes and mouse phenotypes.** The most comprehensive on-line resource linking gene to mouse phenotype is the knockout mouse portion of BioMedNet (Table 4). This site retrieves literature citations following searches by gene, gene function, cellular process, organ system or pathology. The mouse model lists provided by the Jackson Laboratory are organized according to categories of organ system and physiologic processes and provide a good starting point to look for mutant mice falling under these categories. Published descriptions of organ specific mutant mouse pathology can be found in several texts.

**Linking mouse and human phenotypes.** The OMIM website (Table 4) is the most comprehensive source of human gene defects and associated phenotypes. A search by gene or phenotype typically produces a substantial list of results, which usually describe known information on a gene or the phenotype(s) of murine counterparts.

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