The development of new methods for manipulating the mouse genome by transgenic and gene-targeting technologies has dramatically increased our ability to create mouse models for human genetic diseases. These mouse models have greatly facilitated the understanding of the pathogenesis of some human diseases and are beginning to be used in screening of therapeutic agents. In this review, we outline 2 basic techniques that are most frequently used to alter the mouse genetic makeup and summarize their application in the study of some common neurodegenerative disorders.

**BASIC TECHNOLOGIES**

There are 2 basic technologies to generate mouse models: transgenesis and gene targeting. In general, when the disease trait is dominant, i.e., either a gain-of-function or a dominant-negative effect mutation leads to the disease, the mouse model can be established through transgenic methods. When the disease is recessive, i.e., a loss-of-function mutation causes the disease, the mouse model can be developed by a gene-targeting (knockout) strategy. A technique termed knock-in, which is technically similar to knockout, can be used to generate mouse models for either gain-of-function or loss-of-function mutations. In addition, a conditional gene targeting in mice has been recently described by using a Cre/loxP site-specific recombination system.

**TRANSGENESIS**

The introduction of foreign genes into the germ line of mice is a major technological advance in biology and medicine. There are 3 major methods to introduce foreign genes into mice.

Microinjection of DNA

Microinjection of a cloned gene directly into a pronucleus of a fertilized mouse egg has been the most widely used method for generating transgenic mice. Typically, multiple DNA molecules (usually 2 to 40 copies) arranged in a head-to-tail array integrate stably into a single chromosomal site of the mouse genome. The principal advantage of direct microinjection of recombinant DNA into the pronucleus is the high efficiency. Approximately 10% to 30% of the offspring contain the microinjected gene, and generally the transgenic mice express the transgene in a predictable manner in the cells. Another advantage is that DNA fragments or genes of large size, ranging from several kilobases to several hundred kilobases, can be efficiently injected and integrated into the mouse genome. The disadvantage of this approach is that the integration site cannot be predetermined, and, therefore, the expression level of the transgene cannot be predicted and may vary, depending on the site of integration.

Retrovirus Infection

Retroviruses or retroviral vectors can deliver the target DNA into mouse genome by infection of the mouse embryos at either preimplantation or postimplantation stage. The ability to introduce proviruses into the mouse germ line has allowed the production of random mutations. The gene disrupted in these mutants is easy to isolate since the provirus serves as a tag for the locus.
The main advantage of the use of the retroviruses or retroviral vectors for gene transfer into the mouse genome is the technical ease of introducing virus into the embryos at various stages. Furthermore, it has proved much easier to isolate the flanking host sequences of a proviral insert than those flanking a DNA insert derived from pronuclear injection. This is a great advantage when attempting to identify the host gene disrupted by insertion of the proviral DNA. The 2 main disadvantages of the use of retroviruses for gene transfer are the size limitation of the transduced DNA and the uncertainty in reproducibility of expressing the transduced gene in mice.

**Embryonic Stem Cells**

Embryonic stem (ES) cells are established in vitro from explanted blastocysts and retain their normal karyotype in culture. When injected into host blastocysts, ES cells can colonize the embryo and contribute to both the soma and germ line of the resulting chimeric mice. Genes can be effectively introduced into ES cells by DNA transfection or by retrovirus-mediated transduction, and the cells selected for the presence of foreign DNA retain their pluripotent character.

**Basic Procedures**

The main steps in making transgenic mice are illustrated in Figure 1. Four- to 8-week-old female mice are intraperitoneally injected with pregnant mare serum, followed approximately 48 hours later by an injection of human chorionic gonadotropin. The use of pregnant mare serum and human chorionic gonadotropin increases the number of the embryos harvested per female over natural mating. The superovulated female mice are immediately mated with male mice of the same strain. One-celled mouse embryos are collected from the oviducts of the donor females. Females are killed, and oviducts are removed. Oviducts are placed in a medium supplemented with hyaluronidase, which serves to disperse cumulus cells that surround the embryos. The ampulla is torn open to release the embryos. Embryos are subsequently transferred to new media to remove debris and traces of hyaluronidase. Unfertilized or deformed embryos are discarded, and those remaining are cultured in media until ready for microinjection. Using an inverted microscope and with the help of a micro-manipulator, the embryos are held stationary by a holding pipette such that the male pronucleus faces the injection pipette. The zona pellucida, plasma membrane, and male pronuclear membrane are pierced with the injection pipette, and the DNA solution previously loaded into the pipette is injected into the male pronucleus until the pronucleus has swelled to approximately twice its original size. In most cases, the male pronucleus is the target for microinjection mainly because it is larger and, therefore, easier for injection. Pseudopregnant foster mothers are obtained by natural mating of 6- to 12-week-old females with vasectomized males. The females with postcoital vaginal plugs are used as embryo recipients. The embryos that survive microinjection are transferred into the oviducts of the pseudopregnant females using a glass pipette. The transgenic founder mice are identified by either polymerase chain reaction, dot-blot, slot-blot, or Southern blot analysis, using genomic DNA isolated from the mouse tails. Once the transgenic founder mice are identified, they are bred to nontransgenic mice to determine if they are germ line transgenic and also to provide additional animals for further biological and pathological analyses.

**GENE TARGETING**

The methods to produce animal models of recessive genetic diseases are different from those used in studying autosomal dominant diseases. To create an animal model of autosomal recessive disease, both alleles of the normal gene must be inactivated. The technique of gene knockout was developed for this purpose. In the early 1980s, the efficiency of the process used to generate chimeras improved markedly when methods were developed to culture totipotential cells from the inner cell mass of the blastocyst. These pluripotent cells, termed ES cells, can be genetically altered and then microinjected into the cavity of an intact
mouse blastocyst after 3.5 days of gestation. The genetically altered ES cells can populate all the tissues of the developing mouse from the blastocyst stage. The contribution of the ES cells to the genetic makeup of the chimeric animal that develops from the injected blastocyst is most easily assessed by using ES cells and blastocysts whose genes for coat color differ. If the ES cells contribute to the germ cells of the developing mouse embryo, their entire haploid genome can be passed to subsequent generations.

The isolation of ES cell line and the demonstration that transfected DNA can recombine with its homologous chromosome counterpart in the genome in the mammalian cell lines, including ES cells, are the 2 most important observations that led to the production of gene knockout animals. The ES cells are derived from the inner cell mass of a blastocyst and can be kept indefinitely in culture without jeopardizing their totipotency and can be reintroduced into a host embryo by either injection into blastocyst cavity or aggregation of ES cells with morulae. Both methods are successfully used to produce chimeric animals in which manipulated ES cells have contributed to somatic tissue and germ line lineage.

A common approach to disrupt gene function by homologous recombination in ES cells is to construct a vector (targeting vector or knockout vector) designed to undergo homologous recombination with its chromosomal counterpart. The goal of the gene-targeting (knockout) method is to replace a specific gene of interest with another one that is inactive, altered, or irrelevant. Since the integration of the knockout vector into a random chromosomal site is much more frequent than into its homologous site in the genome, several techniques are designed to select cells with site-specific recombinant events. To increase the probability of site-specific replacement, both ends of the replacement gene are flanked by long DNA sequences that are homologous to the sequences that flank the target gene. Gene constructs of this type permit corresponding stretches of the DNA to be exchanged (this is termed homologous recombination) when the DNA breaks and rejoins. The frequency of the homologous recombination is very low. Therefore, there must be a way to select the rare cells in which the target gene has been replaced by the constructed gene. The 2 strategies to select specific cells, positive and negative selection, are illustrated in Figure 2.

Electroporation is the most efficient method for introducing DNA into ES cells. After the targeting construct is delivered into the ES cells, the recombinant cells can be selected based on the targeting construct design. To date, all targeted mutations introduced into the germ line have been created using a selectable marker, usually neomycin resistance and occasionally hygromycin resistance, to disrupt the coding sequence of the targeted gene. To enrich for targeted events, the most widely used strategy is the positive or negative selection approach that can enrich 2- to 100-fold.

In the construct, the bacterial neomycin resistance (neor) gene disrupts the coding sequence of the mouse gene. In addition, the herpes simplex virus thymidine kinase (HSV-tK) gene is placed at one or both ends of the targeting construct. The antibiotic G418 is used to select for cells in which the DNA construct containing the neor gene has been integrated, either randomly or by homologous recombination. The nucleoside analog ganciclovir or 1-(2’-deoxy-2’-fluoro-B-D-arabinofuranosyl)-5-iodouracil is converted by

Figure 2. Steps in gene targeting. ES cells indicates embryonic stem cells; neor, bacterial neomycin resistance gene; and HSV-tK, herpes simplex virus thymidine kinase gene.
Amyotrophic lateral sclerosis is a fatal neurological disease characterized by the degeneration of the motor neurons in the brain and spinal cord. About 5% to 10% of ALS cases are familial (FALS), and the rest are sporadic. In most cases, FALS is inherited as an autosomal dominant trait. Two gene loci for the dominant form of FALS and another 2 gene loci for the less frequent recessive form of FALS have been identified. In addition, a dominant form of juvenile ALS-like syndrome has been mapped to 9q34. The copper-zinc superoxide dismutase (SOD1) gene on chromosome 21 is the only identified genetic element that when mutated is causative of FALS. Mutations in SOD1 are found in about 20% of FALS cases. SOD1 converts superoxide to form molecular oxygen and hydrogen peroxide, the latter of which is detoxified by catalase and glutathione peroxidase. Because both superoxide and hydrogen peroxide are toxic to cells, it is speculated that the disturbance of free radical homeostasis, by either an increase or decrease in dismutase activity, may lead to cell death. Initially, when decreased dismutase activity was identified in individuals with mutant SOD1, it was hypothesized that the decrease rather than increase in dismutase activity may be responsible to the disease. Although several lines of evidence supported this hypothesis, transgenic mouse overexpressing the mutant SOD1 developed an ALS-like syndrome, which strongly argues for a gain-of-function mechanism.

The first mutant transgenic mouse line was developed by overexpression of G93A mutation, a mutation that causes FALS in humans. The mouse expressing high levels of mutant SOD1 (G93A) developed a phenotype and pathologic condition similar to human ALS, whereas the mice overexpressing wild-type SOD1 remained unaffected. This observation was soon confirmed by other groups. Wong et al reported that transgenic mice that overexpressed G37R mutation of SOD1 developed severe, progressive motor neuron disease. The most obvious cellular abnormality in G93A mice and the G37R mice is the presence of membrane-bound vacuoles in axons, dendrites, and cell bodies. This pathological aspect is not a common feature of human disease, but ALS mice expressing the lower amount of mutant SOD1 have an abnormality akin to that seen in humans. Ripp et al produced another transgenic mouse line by introducing the mouse SOD1 mutation G86R, corresponding to G85R in humans. The G86R mice also showed degenerative changes of motor neurons within the spinal cord, brainstem, and neocortex. Common to all 3 lines of mutant SOD1 transgenic mice is that the mice with the higher copy number of the mutant SOD1 gene and with higher SOD1 activity become paralyzed earlier, providing strong evidence that onset of disease is influenced by dose of mutant SOD1 protein and that decreased SOD1 activity is not the primary care of ALS. Further support of this observation comes from SOD1 knockout mice. Reaume et al generated SOD1 knockout mice using homologous recombination. They found that mice lacking SOD1 developed normally and that 1-year-old mice show no overt motor deficit. These mice, however, do have increased motor neuron loss after axonal injury and distal axonal loss with age. These results indicate that SOD1 is not necessary for normal motor neuron development and function but may be required in physiologically stressful conditions or those following injury.

The mechanism for mutant SOD1-mediated FALS remains unknown. Numerous hypotheses have been proposed and some tested in ALS mice.

**Apoptosis**

By crossbreeding mice that overexpress bcl-2 to SOD1-G93A transgenic mice, Kostic et al found that overexpression of the antiapoptotic protein bcl-2 delayed onset of ALS and prolonged survival in G93A mice, although the duration of the disease was not altered. Friedlander et al crossed SOD1-G93A mice to transgenic mice that overexpressed a dominant negative inhibitor of the interleukin 1β-converting enzyme (ICE), which has the active-site Cys substituted for a Gly, in neurons under the control of a neuron-specific enolase promoter (NSE-M17Z). They
found that although the timing of disease onset in both the G93A mice and ICE (M17Z)/G93A mice was not different, the double transgenic mice survived significantly longer than the G93A mice. This finding was further expanded by Pasinelli et al.11 By using transgenic mice and cell culture systems, they found that caspase-1 is activated in neurons expressing mutant SOD1 protein. Proteolytic processing characteristic of caspase-1 activation is seen both in spinal cords of transgenic ALS mice and neurally differentiated neuroblastoma cells with SOD1 mutations. This activation of caspase-1 is enhanced by oxidative challenge, which triggers cleavage and secretion of the ICE substrate, pro–interleukin 1β, and apoptosis. However, evidence of lack of apoptosis in mutant SOD1 mice with ALS was also reported.12

Inclusions

Cytoplasmic Lewy body–like hyaline inclusions are found in motor neurons of patients with ALS. In familial cases with the SOD1-A4V mutation, the inclusions are intensively SOD1 positive by immunohistochemistry. The SOD1-positive inclusions were also identified in transgenic mice expressing SOD1-G93A, raising the possibility that the formation of these inclusions may interfere in the neuronal cell function and lead to motor neuron death in FALS. By crossingbreeding transgenic mice that express SOD1-G85R to SOD1-deficient mice, Bruijn et al13 developed mouse lines that express SOD1-G85R transgenic mice with or without expression of mouse endogenous SOD1. They found that the presence of SOD1-positive aggregates was a common finding of the disease regardless of the presence or absence of endogenous SOD1. We have recently made a new transgenic mouse model that expresses truncated SOD1 (SOD1-L126Z) (H.-X.D. and T.S., unpublished data, October 1999). These mice develop the typical phenotype and pathologic features of ALS. SOD1 inclusions can be detected in neurons and neuritic processes in the gray matter of the spinal cord and brainstem, suggesting that an altered form of mutant SOD1 may participate in the pathogenesis of FALS. This mouse probably provides the minimal lesion necessary for neurodegeneration and provides evidence that a truncated SOD1 polypeptide is sufficient to cause disease. These results lead to the question of what is the minimum fragment of SOD1 that can cause ALS.

Neurofilaments

Neurofilaments are another suspected component involved in the pathogenesis of ALS. It was reported that overexpression of neurofilament subunits NF-L or NF-H in transgenic mice produced morphologic alterations that resemble the pathologic features of human ALS, although without extensive motor neuron death. Mutations in NF-H have also been described in some cases of ALS, although negative results have also been reported. To investigate how neurofilaments might cause neuroopathy and its relevance to ALS, several groups used transgenic mouse models and reported some interesting results. Eyet et al14 crossed SOD1-G37R mice with mice overexpressing NF-β-galactosidase fusion protein (neurofilaments are absent in the axons of these mice because they are trapped in the neuron cell bodies) and showed that axonal neurofilaments play no essential role in SOD1-mediated neuronal degeneration. Williamson et al15 mated SOD1-G85R mice to NF-L knockout mice to produce SOD1-G85R mice with NF-L deficiency. They found that the complete absence of NF-L slowed the onset of SOD1-G85R–mediated ALS, and this slowing correlated with increased levels of NF-M and NF-H subunits in the cell bodies. Couillard-Despres et al16 demonstrated that mice overexpressing both SOD1-G37R and NF-H subunits have increased mean lifespan by 65% in comparison to mice expressing SOD1-G37R only. Taken together, these results indicate that although disorganized neurofilaments can sometimes cause neuropathy, neurofilaments are not required for mutant SOD1-mediated neurodegeneration; rather absence of neurofilaments from the axons may play a protective role in this process.

GLUTAMINE EXPANSION DISORDERS

Expansion of CAG triplets (coding for glutamine repeats) within the coding regions of several genes leads to specific autosomal dominant neurodegenerative disorders, such as HD, and several SCAs (SCA1, SCA2, SCA3, SCA6, and SCA7), dentatorubral-pallidoluysian atrophy, and X-linked recessive spinobulbar muscular atrophy (Kennedy disease). Several findings make this group of diseases unique. First, these disorders are characterized by “anticipation.” In successive generations of a pedigree, the severity of the disease increases and age of onset of the clinical symptoms decreases. Second, the age of onset may be inversely correlated with the number of CAG repeats. Third, these genes are not homologous except that they all share CAG expansion. Even in SCAs, the genes are not homologous, causing different SCA subtypes. Fourth, although the expression of the genes is widespread, each disorder has highly selective but overlapping degeneration of different neurons, such as striatal neurons in HD, cerebellar Purkinje cells in SCAs, and brainstem and spinal motor neurons in spinobulbar muscular atrophy.

The mechanisms by which the CAG expansions cause these glutamine expansion disorders are not known. In the past several years, transgenic studies have provided substantial information in this field. Herein we describe several representative experiments in HD and SCA1 research as examples.

Huntington disease is a devastating neurologic disorder associated with progressive chorea, rigidity, and dementia. It usually manifests in midlife and results in selective neuron loss that is most prominent in the striatum and basal ganglia. The HD gene encodes a ubiquitously expressed protein (huntingtin) containing a glutamine repeat. The number of repeats varies from 8 to 35 in healthy individuals. Individuals develop HD if the repeat number is more than 36. To understand the normal function of the HD gene, 3 groups have independently created knockout mice using homologous recombination. They found that homozygous knockout mice showed developmental retardation and died around 8 days. The heterozygous mice displayed increased motor activity and cognitive deficits. Because neither homozygous nor het-

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erozygous HD knockout mice recapitulate the phenotype and neuropathologic features of HD, it follows that the HD gene, although essential for postimplantation development and normal functioning of the basal ganglia, does not cause HD due to loss of the intrinsic function. A gain-of-function mechanism is therefore postulated for the pathogenesis of HD.16,17

To investigate the role of glutamine expansion of huntingtin in HD, Mangiarini et al18 established a transgenic mouse model using a construct that contains a 1.9-kilobase human genomic fragment, including promoter, exon 1 with approximately (CAG)130, and first 262 base pairs of intron 1. The transgenic mice exhibited a progressive neurological phenotype that resembles many of the features of HD, including choreiform-like movements, involuntary stereotypic movements, tremor, and epileptic seizures. Because this transgene only expressed a small, glutamine-containing portion of huntingtin and still led to the disease, it was suggested that expression of a truncated amino-terminal fragment of huntingtin–induced cell death. Conversely, suppression of the huntingtin gene to direct neurodegeneration seen in HD, they found that when cultured striatal neurons were transfected with complementary DNAs (cDNAs) coding wild-type or mutant versions of huntingtin, mutant huntingtin induces neurodegeneration by an apoptotic mechanism. Blocking nuclear localization of the mutant huntingtin–induced cell death, suggesting NII formation may correlate with huntingtin-induced cell death. NIIs and to induce neurodegeneration. The ataxin-1 (82) with K772T is expressed in Purkinje cell nuclei. These mice developed ataxia and Purkinje cell abnormalities similar to the original SCA1 mice. However, no evidence of NII was found. Thus, they concluded that although nuclear localization of ataxin-1 is necessary for the development of the disease, NII of ataxin-1 are not required to initiate the pathogenesis in the transgenic mice. Similar results were also obtained by Saudou et al.22 By using a cellular model that recapitulates features of neurodegeneration seen in HD, they found that when cultured striatal neurons were transfected with complementary DNAs (cDNAs) coding wild-type or mutant versions of huntingtin, mutant huntingtin induces neurodegeneration by an apoptotic mechanism. Blocking nuclear localization of the mutant huntingtin suppressed its ability to form NII and to induce neurodegeneration. However, the formation of NII did not correlate with huntingtin-induced cell death. Conversely, suppression of the NII formation resulted in an increase in mutant huntingtin–induced cell death, suggesting NII formation may reflect a cellular mechanism to protect against huntingtin-induced cell death. Recently, Cummings et al23 presented evidence that although the pathogenic role of small, submicroscopic aggregates still remains possible, the large inclusions are not required for expanded polyglutamine pathologic.

ALZHEIMER DISEASE

Alzheimer disease is the fourth leading cause of death in the United States. Two histopathologic hallmarks of AD are the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles, which consists primarily of amyloid β (Ab) and abnormally phosphorylated tau protein, respectively. About 10% of AD cases are familial and show autosomal dominant inheritance, and mutations in 3 genes (amyloid precursor protein [APP] and presenilin 1 and 2 [PS1 and PS2]) have been described. The presence of the apolipoprotein E4 (apoE4) allele is a robust risk factor for late-onset AD.

Amyloid Precursor Protein

After the identification of the pathogenic mutations in the APP gene, many investigators tried to develop a mouse model. Partly due to the technical difficulties in handling the large APP gene (approximately 400 kilobases), transgenes were made with APP cDNA sequences and a variety of promoters. Unfortunately, these transgenic mice failed to show extensive type neuropathology and phenotype. In 1995, Games et al24 constructed a hybrid transgene, which includes the full-length human APP cDNA with Val171Phe mutation under the control of the platelet-derived growth factor promoter. Inserted into this transgene are introns 6, 7, and 8 of APP gene, which are important for alternative splicing of the gene product. Probably due to these changes, this construct drives much higher levels of APP expression than standard cDNA constructs. The resulting transgenic mice developed many of the pathological hallmarks of AD, including numerous extracellular thioflavin S–positive Ab deposits, neuritic plaques, synaptic loss, astrocytosis, and microgliosis. Using the regulatory elements of the PpP gene to direct neuronal expression, Hsiao et al25 established transgenic mice overexpression APP containing L670, M671I. They
found that the behaviorally deficit transgenic mice had differentially increased Aβ, with a 5-fold increase in Aβ40 but a 14-fold increase in Aβ42(43). Although there is no obvious occurrence of neurofibrillary tangles in either V717F or L670N, M671L mice, these results indicate that APP/Aß may play a primary role in the pathogenesis of AD.

PS1 and PS2

PS1 and PS2 encode membrane-associated proteins of great similarity; therefore, they may share similar function. Mutations in the PS1 gene account for most early-onset familial AD. To understand the normal function of PS, Shen et al²⁶ created a PS1 gene knockout mouse model. The homozygous mutants died shortly after birth and showed multiple defects in skeleton and central nervous system, suggesting that PS1 is essential for proper formation of the axial skeleton, normal neurogenesis, and neuronal survival. Using platelet-derived growth factor β2 promoter to drive the neuronal expression, Duff et al²⁷ developed several lines of transgenic mice that overexpressed mutant PS1 with M146L or M146V. They found that expression of mutant PS1 selectively increase Aβ(43), suggesting that PS1 mutations cause AD through a gain of deleterious function that increases the amount of Aβ(43) in the brain. This finding was confirmed and further expanded by in vivo and in vitro experiments. By coexpression of human PS1 or PS2 and human Aβ precursor in cell lines and transgenic mouse models, Citron et al²⁸ quantitatively analyzed the effects of PS expression on APP processing. They found that in both model systems, mutant but not the wild-type PS significantly increased the production of Aβ42. They also suggested this increased production of Aβ42 be mediated by alteration of the activity of γ-secretase but not α- or β-secretase. To investigate the possible interaction between APP and PS, Holcomb et al²⁹ developed double transgenic mice that carried both mutant APP and PS1 transgenes. They found that double transgenic mice developed large numbers of fibrillar Aβ deposits in cerebral cortex and hippocampus far earlier than their single transgenic littermates, suggesting an enhancing effect of mutant PS1 on Aß plaque formation of mutant APP transgenic mice.

Recently, Zhang et al³⁰ found that PS1 forms a complex with β-catenin in vivo that increases the stability of β-catenin. Pathogenic mutations in the PS1 gene reduces the ability of PS1 to stabilize β-catenin and lead to an increased degradation of β-catenin in the brains of transgenic mice that overexpress PS1 with H163R or I143T. Loss of β-catenin signaling increases neuronal vulnerability to apoptosis induced by Aβ protein. Therefore, they proposed that mutations in PS genes increase neuronal apoptosis by altering the stability of β-catenin, predisposing individuals to early-onset AD.

Apolipoprotein E

Apolipoprotein E (apoE) is a 34-kd, glycosylated, lipid-binding protein that mediates the redistribution of lipids among cells. It is highly expressed in brain and liver. Human apoE exists in 3 major isoforms encoded by distinct alleles (E2, E3, and E4) on chromosome 19. Clinical evidence suggests that besides age, the apoE4 is the most important known risk factor for the development of late-onset familial and sporadic forms of AD, whereas E2 provides relative protection from this illness. Although many hypotheses have been proposed, it remains unclear how apoE4 affects cognition and increases the AD risk. To examine the effects of apoE4 on AD, several groups have made apoE knockout mice and transgenic mice expressing different alleles of apoE. By crossbreeding, mice expressing different human apoE isoforms in the absence of mouse endogenous apoE have been established. Although these mice did not show evidence of senile plaques, some mouse lines showed impairment in learning and in vertical exploratory behavior.

PRION DISEASES

The prion diseases, sometimes referred to as the transmissible spongiform encephalopathies, include kuru, Creutzfeldt-Jakob disease, and Gerstmann-Strassler-Scheinker disease of human as well as scrapie and bovine spongiform encephalopathy of animals. The unique characteristics of these diseases for many decades, most solid lines of evidence regarding the pathogenesis are derived from transgenic studies of the inherited form of the diseases.

The discovery of a mutation at codon 102 (P102L) in the PrP gene in a family with Gerstmann-Strassler-Scheinker disease established the genetic basis for prion diseases. To investigate the nature of the diseases, Hsiao et al³¹ and Telling et al³² created a transgenic mouse model that overexpressed a mutant mouse PrP with a substitution P101, which corresponds to P102L in human mutant PrP identified in Gerstmann-Strassler-Scheinker disease. The resulting mice spontaneously developed spongiform neurodegeneration between 50 and 410 days of age, while the control lines that overexpressed wild-type PrP remained normal for more than 600 days. Furthermore, they found that when Syrian hamsters or mice were inoculated with brain homogenates from these spontaneously sick transgenic mice, the inoculated animals developed similar disease. However, the control group that was inoculated with brain homogenates from normal mice remained free of symptoms. These findings indicate that mutant PrP gene not only causes but also produces infectious particles for prion disease.

If prion diseases are caused exclusively by prion and not any other agents such as DNA and RNA, the next question would be how prion propagates and what are the substrates for the prion propagation. The “protein only” hypothesis proposes that PrPSc (prion protein: scrapie form) is the pathogenically modified form of PrPC (prion protein: cellular form) and PrPSc is devoid of nucleic acid. PrPSc converts PrPC to PrPSc when PrPSc directly contacts PrPC. If so, the prediction would be that an animal devoid of PrPC should be resistant to PrPSc and not susceptible to prion disease. To test this hypothesis, Bueler,³³ Manson,³⁴ and Sakaguchi³⁵ and their coworkers have generated 3 independent lines of PrPC knockout mice by...
homologous recombination using different strategies. These PrPC-free mice developed normally and showed no obvious pathological phenotype by a certain age. When these mice were inoculated with mouse prion, the mice remained healthy and prions failed to amplify in mouse brain, while the wild-type controls died from the disease. However, when PrP transgene was introduced into PrPc knockout mice, the susceptibility to prion disease was restored. The susceptibility seems PrPC dose related, because heterozygous mice that express PrPC at about half the normal level showed less susceptibility than wild-type controls.

To identify the regions of PrP related to prion propagation and scrapie pathogenesis, Shmerling et al developed PrP knockout mice expressing PrPs with amino-proximal deletions. They found that PrP with deletions of residues 32-121 or 32-134, but not with shorter deletions, caused severe ataxia and neuronal death limited to the granular layer of the cerebellum as early as 1 to 3 months after birth. The defect was completely abolished by introducing 1 copy of a wild-type PrP gene. Shmerling et al speculated that these truncated PrPs are nonfunctional and compete with some other molecule with a PrP-like function for a common ligand.

CONCLUSIONS

The genetic mouse models of neurodegenerative disease have been very instructive and full of surprises. The ALS model overturned the loss of function hypothesis and provided the lead in development of mouse models of dominantly inherited neurodegenerative diseases. More importantly, as an easily measurable phenotype, it provides a tool for screening of preventive and therapeutic agents for neurodegeneration. The AD mouse model demonstrates the limitation of models of cognitive impairment, and the measurement in those has to rely on pathology. The CAG expansion models teach us the primary role of the glutamine repeat and allow for the examination of the role of intranuclear aggregates in pathogenesis. The prion model is instructional in the role of the intrinsic prion gene, since prion disease is not one of instructive particles multiplying but one of conversion of endogenous prion to toxic form(s). However, the central problem of the complex mechanisms by which neurons degenerate in these disorders still remains to be answered in the future.

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