TRANSGENIC ANIMALS

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DEPARTMENT OF BIOTECHNOLOGY
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New and Improved Animals

- Every cell carries new genetic information
  - Into germline not just some somatic cells (gene therapy)

- Novel genetic information consists of genes transferred from other organisms
  - Transgenes
    - Engineered before being inserted into host animal
  - Derived from same species, distant relative, or unrelated organism

Creating Transgenic Animals

- Nuclear Microinjection

1. Transgene injected in fertilized egg cells
   - Pronuclei will fuse after DNA is injected into male pronucleus
   - 5-40% success rate
2. Eggs kept in culture during first divisions of embryonic development
3. Embryos are implanted into foster mother
4. Transgenes stably maintained in founder animal
   - Single copy of transgene on one chromosome
   - Heterozygous for the transgene

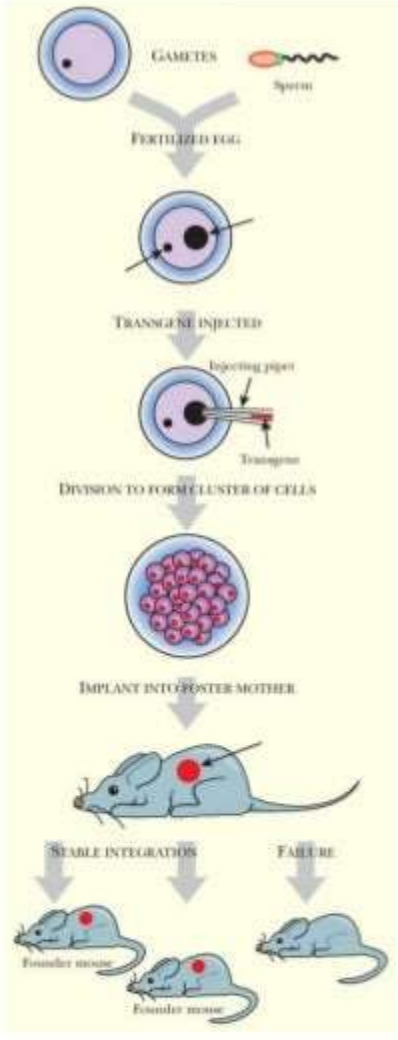

FIGURE 15.1

Creation of Transgenic Animals by Nuclear Injection
In vitro fertilization is used to start a transgenic animal. Harvested eggs and sperm are fertilized, and before the pronuclei fuse, the transgene is injected into the male pronucleus. The embryo continues to divide in culture and is then implanted into a mouse. The "foster mother” mouse has been treated with hormones so that she accepts the embryo and carries on with the pregnancy. The offspring are screened for stable integration of the transgene. Founder mice have one copy of the transgene.

Larger Mice

- 1982: somatotropin gene of rats was cloned and inserted into fertilized mouse eggs
  - Genetically engineered mice were larger
  - First case of gene transferred that was not stably inherited but still functioned
- Promoter from unrelated mouse gene metallothionein
  - Normally expressed in the liver
  - Rat somatotropin usually made in the pituitary gland
- Recombinant human somatotropin (rHST)
  - Used to treat hormone-deficient type of dwarf
  - Receptor-deficient dwarfs cannot be treated yet
- Genetically engineered mice
  - Marathon Mouse, Mighty Mouse, Fierce Mouse, Smart Mouse, K14-Noggin

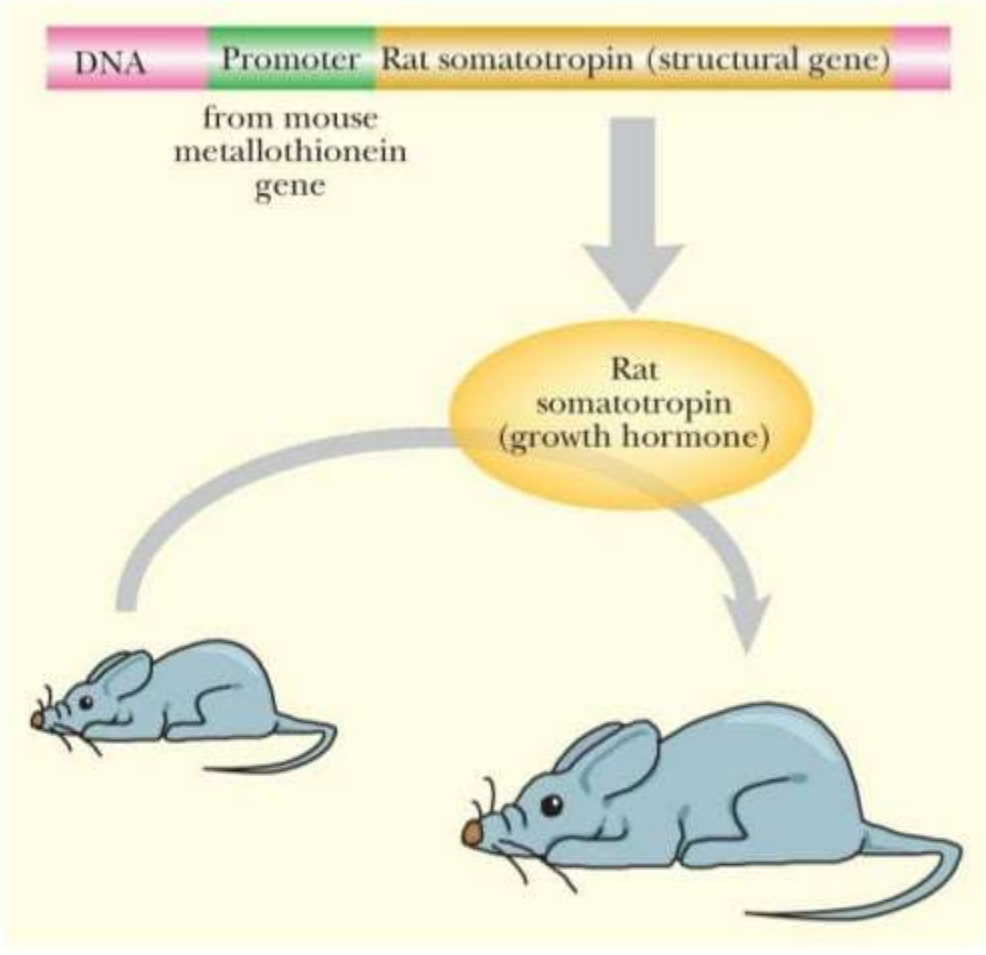

FIGURE 15.2

Large Transgenic Mice
A DNA construct containing the rat somatotropin gene under the control of the mouse metallothionein promoter was used to make a transgenic mouse. The transgene cause the mouse to grow to twice its normal body size.

Recombinant Protein Production

- Cloned somatotropin gene from cows expressed in bacteria
  - Large amounts of the hormone produced
- Recombinant bovine somatotropin (rBST)
  - Diary industry to increase milk production
  - Hormone injection does not produce giant cows
- *E. coli* are cultured to make recombinant proteins
  - Human insulin
  - Expensive and require highly trained workforce
- Livestock to express products may be cheaper
  - 10,000 quarts of milk per cow per year
  - Milk processing and collection industry already exists
- Transgenic goats fro small-scale production
  - Recombinant tissue plasminogen activator (rTPA)
  - Dissolving blood clots

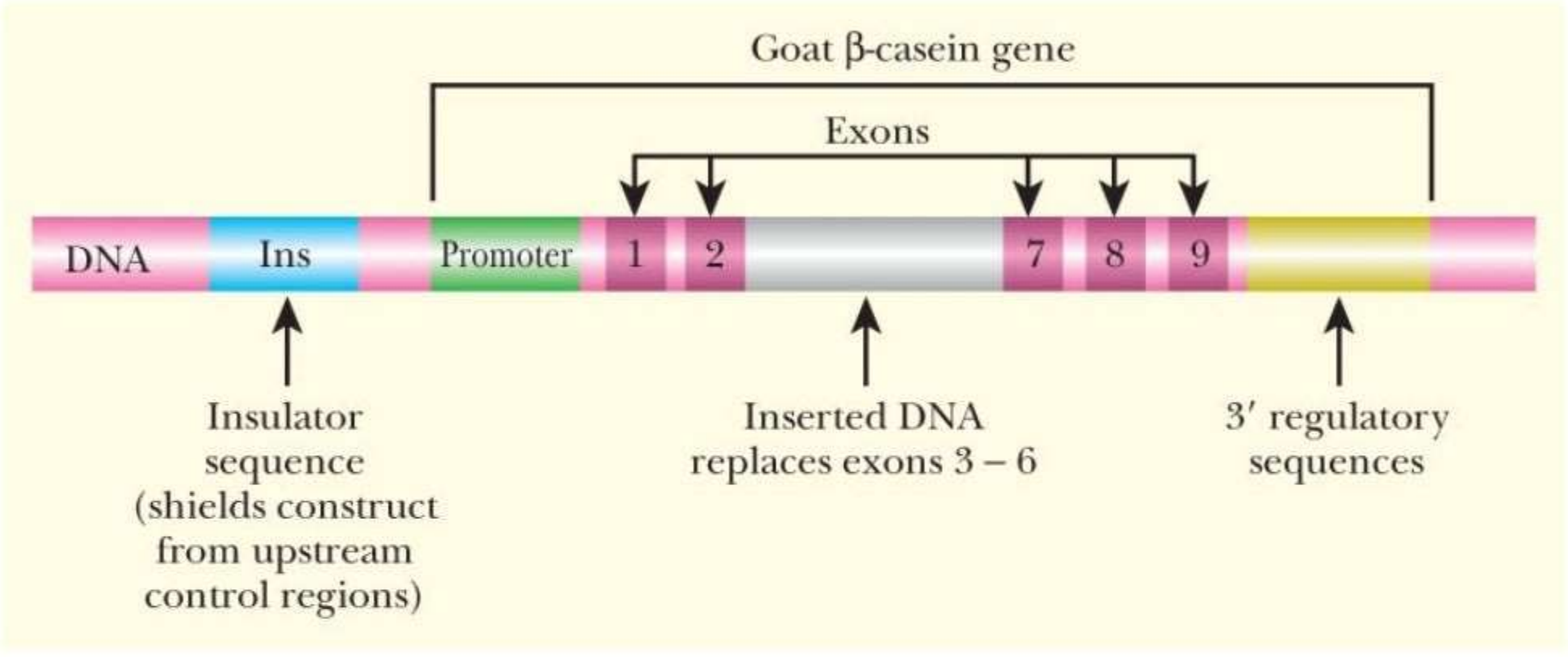

FIGURE 15.3

Milk Expression Construct for Transgenic Goats
In order to express a recombinant protein in goat milk, the gene of interest is inserted in place of the β-casein gene. The transgene will be expressed using the endogenous promoter and 3' regulatory elements that restrict β-casein expression to goat milk. The construct also has insulator sequences that block other regulatory elements from affecting expression (see later discussion).

Knockout Mice

- Widely used in medical research to investigate gene function
  - Knockout mice have selected genes inactivated
- Used for genetic analysis of inherited diseases and cancer
- Knock out gene of interest to study the defects it causes
  1. Target gene is cloned
  2. Gene is disrupted by inserting a DNA cassette into its coding sequence
  3. Inserted DNA segment prevents correct protein synthesis
  4. Inactive gene copy is reinserted into animal
  5. Incoming disrupted gene replaces original, functional copy by homologous recombination
  6. Founder mice are breed together with both copies to produce knockout mice

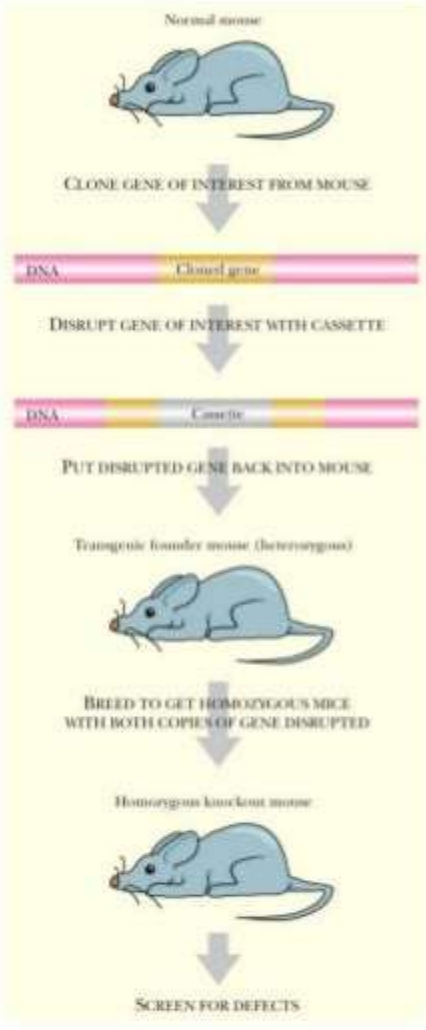

FIGURE 15.4

Knockout Mice

Like traditional transgenic mice, knockout mice are generated in vitro. The target gene is cloned and disrupted by inserting a DNA cassette. This work is usually done in bacteria. Once the construct is made, it is put back into a mouse by injection into the male pronucleus during fertilization (or by other methods outlined later). After the transgenic offspring are born, two heterozygotes are crossed to create a homozygous knockout mouse. These are then screened for defects due to inactivation of the target gene.

Engineered Retroviruses

- Retrovirus vectors that insert into the host chromosomes
  - Introduce DNA and transgenes
  - Can infect embryonic stem cells
- Advantages
  - Single copy of retrovirus plus transgene integrated into genome
  - Does not require skill in microinjection
  - Partial transgenic tissue can be compared to normal tissue on same animal
- Disadvantages
  - Virus DNA is introduced
  - Can only carry limited amounts of DNA
  - Founder animals are always chimeras
  - Rarely used to create a fully transgenic animal

Embryonic Stem Cells

- Derived from the blastocyst
  - Retain ability to develop into any body tissue, including the germline
  - DNA can be introduced as for any cultured cell line
  - Must be maintained under conditions that avoid differentiation
- Engineered embryonic stem cells are inserted into central cavity at blastocyst stage
  - Mixed embryo that is a genetic chimera
  - Some transgenic tissues and others are normal
- Different genetic lines with different fur colors yields a patchwork coat
  - Transgenic sectors of the animal can identified
- Chimeric founder must be mated with a wild-type animal
  - Male chimeras can father many offspring when crossed with WT females

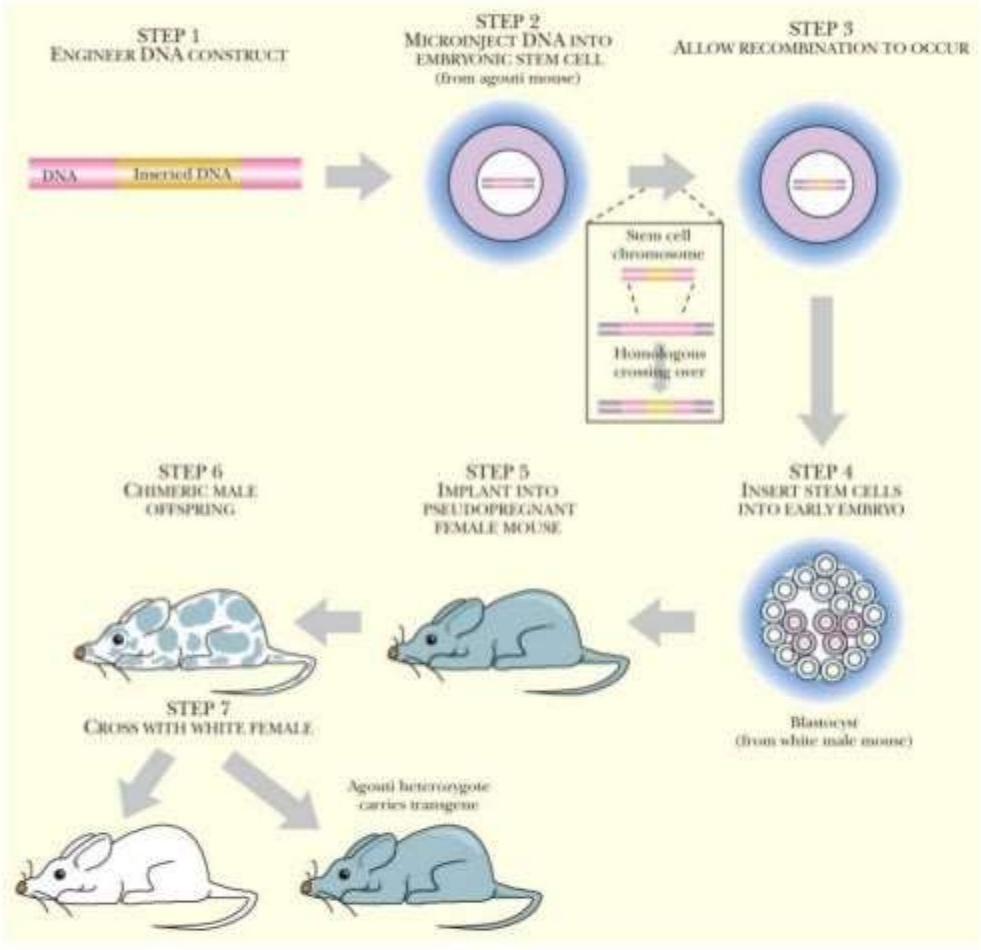

FIGURE 15.5

Use of Embryonic Stem Cells

To create a transgenic animal with embryonic stem cells, the transgene must first be inserted into these cells. The stem cells shown here are from an agouti mouse, that is, a mouse with grizzled brown fur. The stem cells are transformed with the transgene, which integrates by homologous recombination. Then the stem cells are injected into an early male embryo (blastocyst) from a white mouse. The embryo is put into a pseudopregnant female mouse. The offspring are chimeras because the majority of cells in the injected blastocyst are normal. The chimera will have a white coat with patches of brown derived from the injected stem cells. The chimera is crossed with a white female, and any fully brown (agouti) offspring will have the transgene incorporated into the germline.

Location Effects on Expression

- Transgenic animals with same inserted transgene differ in expression
  - location of the transgene
  - nearby regulatory elements in host chromosome
  - Physical state of DNA
    - Regions consisting of large amounts of heterochromatin
    - Tightly packed DNA regions
    - Methylated
    - Coverage of nonacetylated histones

- Position effects confirmed by extracting transgene DNA
  - Extracted from a transgenic animal in which the transgene did not express
  - DNA used to construct another line of transgenic animals
  - If new animals show proper expression then the gene itself is intact
  - Failure due location in original host

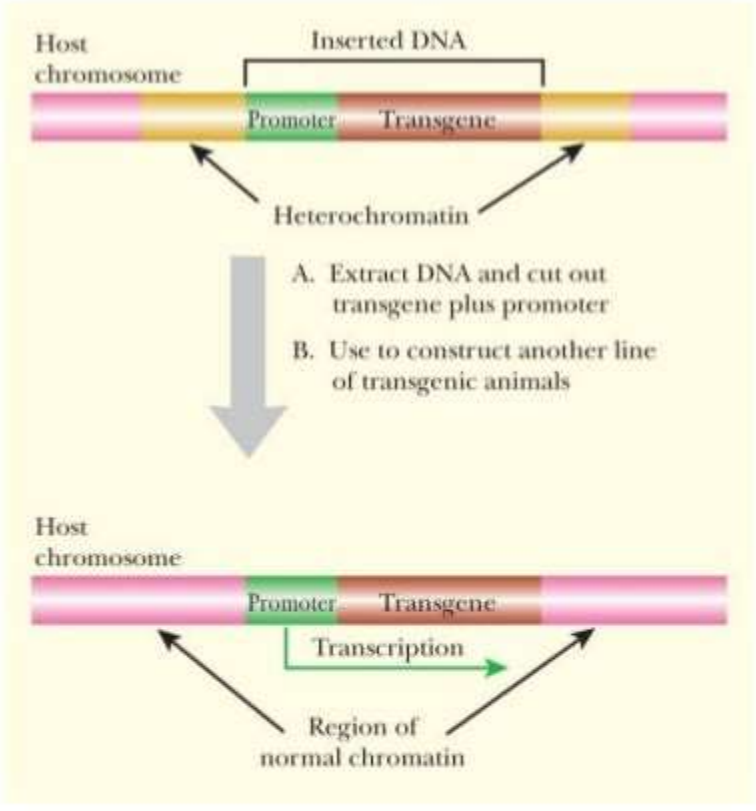

FIGURE 15.6

Failed Expression Due to Transgene Location
DNA carrying a transgene was inserted to generate a transgenic animal. In this instance, the DNA was inserted into a region of heterochromatin. Even though transgenic animals were obtained, the transgene was not expressed. The inserted DNA was removed and used to make another transgenic animal. The transgene was expressed in the second animal, showing that it was intact in the first transgenic animal. Because the location of integration was different, the earlier lack of expression must have been due to a position effect.

Combating Location Effects

- Further engineering to protect against positional effects
  - Inserting appropriate regulatory sequence next to the transgene
- Avoided by targeting the transgene to a specific site
- Regulatory elements can be built in to the transgene construct
  - Dominant control elements
  - Insulator sequences or boundary elements
  - Natural transgenes
- Cloning and manipulating full-length animals genes is inconvenient
  - Excessive lengths of DNA
  - Carry genes on artificial chromosomes
  - YACs used to study long-range regulatory elements and humanized monoclonal antibodies

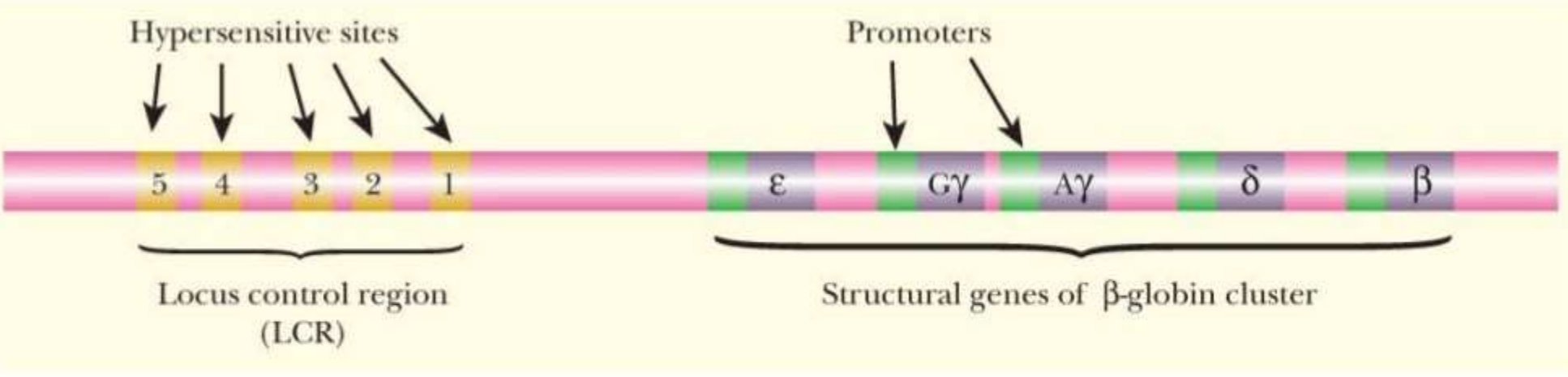

FIGURE 15.7

Locus Control Region (LCR)
The LCR of the β-globin gene cluster enhances expression of all five genes. This control region is outside the individual promoters. The LCR has five DNase I hypersensitive regions, which have multiple consensus sequences for transcription factor binding sites.

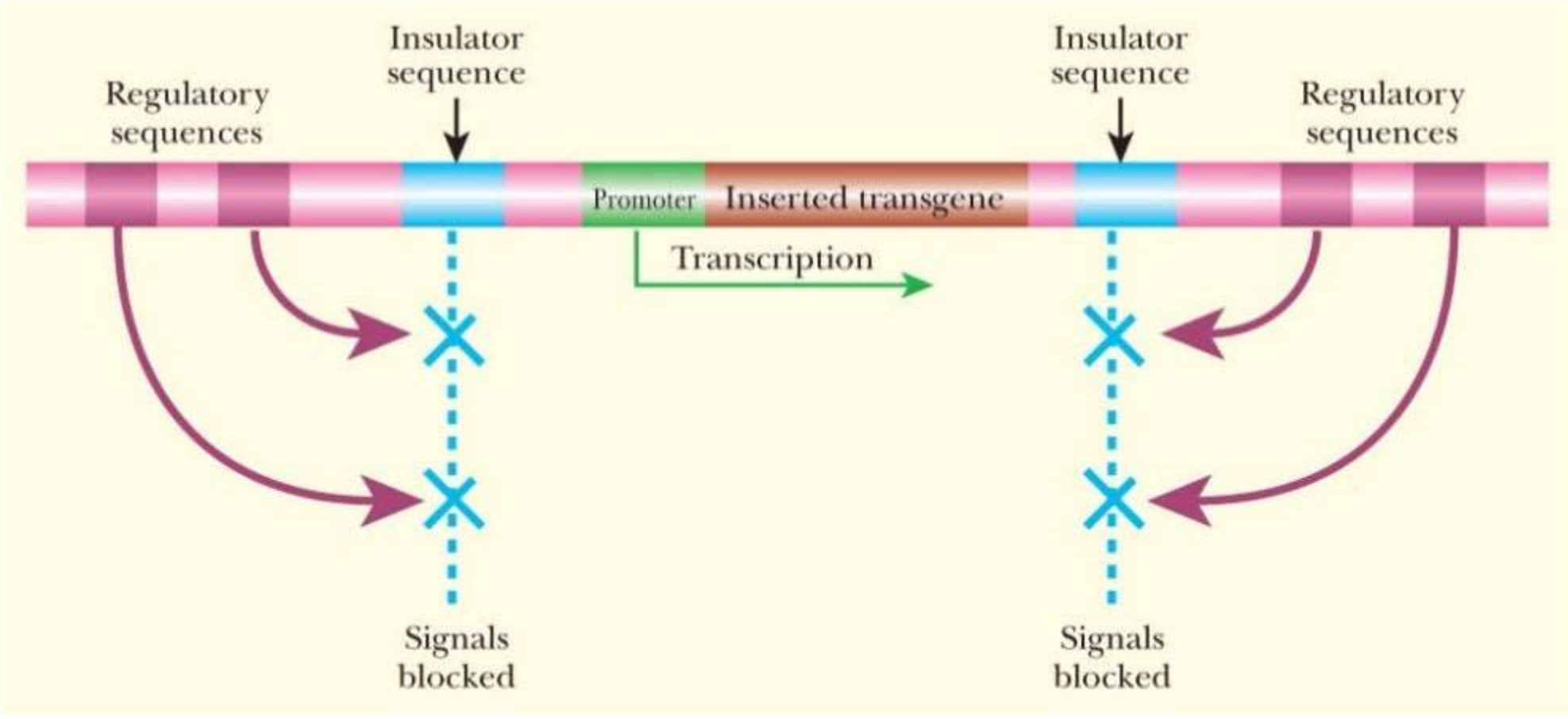

FIGURE 15.8

Protection of a Gene by Insulator Sequences
Insulator sequences are placed flanking a transgene. These protect the transgene from regulatory sequences outside the insulator sequences.

Targeting the Transgene

- Targeting vectors use homologous recombination to insert transgenes at specific locations in the host genome
  - As opposed to random integration with injected DNA
- Chromosomal location effects transgene expression
- The transgene is not necessarily a novel gene
  - Objective to replace original version with an altered version
  - Incoming gene is inserted into same location under same regulation
- Targeting vectors direct integration
  - Inserted DNA is flanked by homologous sequences
  - Linearized just before transforming DNA into cell to promote more efficient recombination
  - Integration may be selected by antibiotics

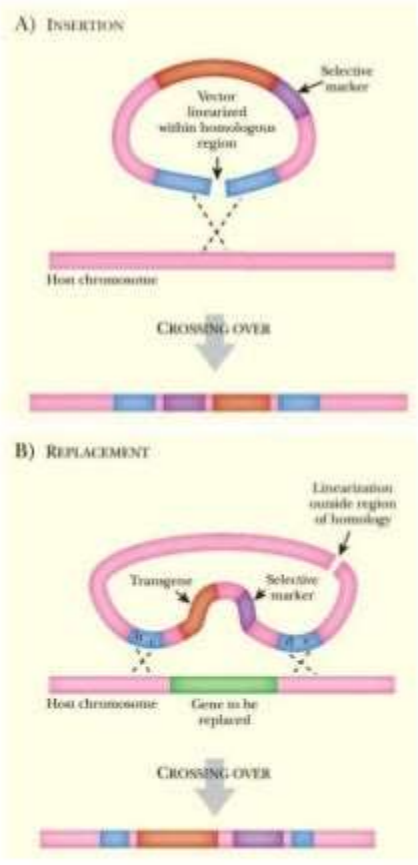

FIGURE 15.9

Targeting Vectors Rely on Homologous Recombination
(A) Targeting vectors can insert a transgene into a host chromosome at a specific location. The vector has sequences (blue) homologous to the insertion point on the host chromosome (pink). The linearized vector triggers a single crossover, thus integrating the transgene and selective marker into the host chromosome. (B) Some targeting vectors promote gene replacement. These vectors have two separate regions flanking the transgene that are homologous to the host. When the linearized vector enters the nucleus, homologous regions align, and crossovers occur on each side of the transgene. The host gene is replaced with the transgene and selective marker.

Deliberate Control

- High-level transgene expression is preferred for industrial production
  - Some proteins are toxic at high amounts
  - Gene expression must be kept low while establishing transgenic animal line
  - Switch gene expression on/off for functional analysis

- Inducible endogenous promoters
  - Natural promoters from the host animal respond to certain stimuli
  - Examples
  - Drawbacks

- Recombinant promoter systems
  - Bacterial repressors used to control transgenes including LacI and TetR

- Transgene regulation via steroid receptors
  - Lipophilic and penetrate cell membranes rapidly
  - Bind protein receptors; bind directly to DNA; regulate gene expression
  - Eliminated within a few hours
  - Must avoid inducing other host genes that respond to steroids

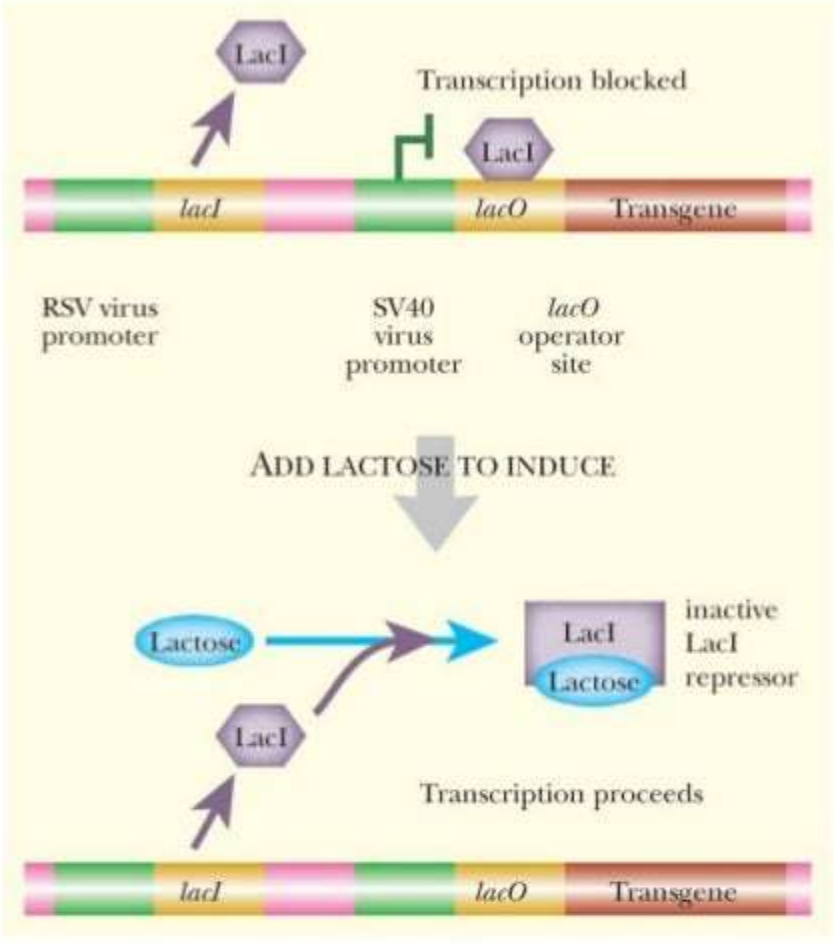

FIGURE 15.10

LacI Control of a Transgene
A Rous sarcoma virus (RSV) promoter drives transcription of the lacI gene. When the construct is expressed in a transgenic animal, LacI protein is produced. The LacI repressor binds to the operator site upstream of the transgene and blocks expression. When the inducer IPTG is added, it binds to LacI, which falls off the operator site. The transgene is then transcribed by the SV40 promoter.

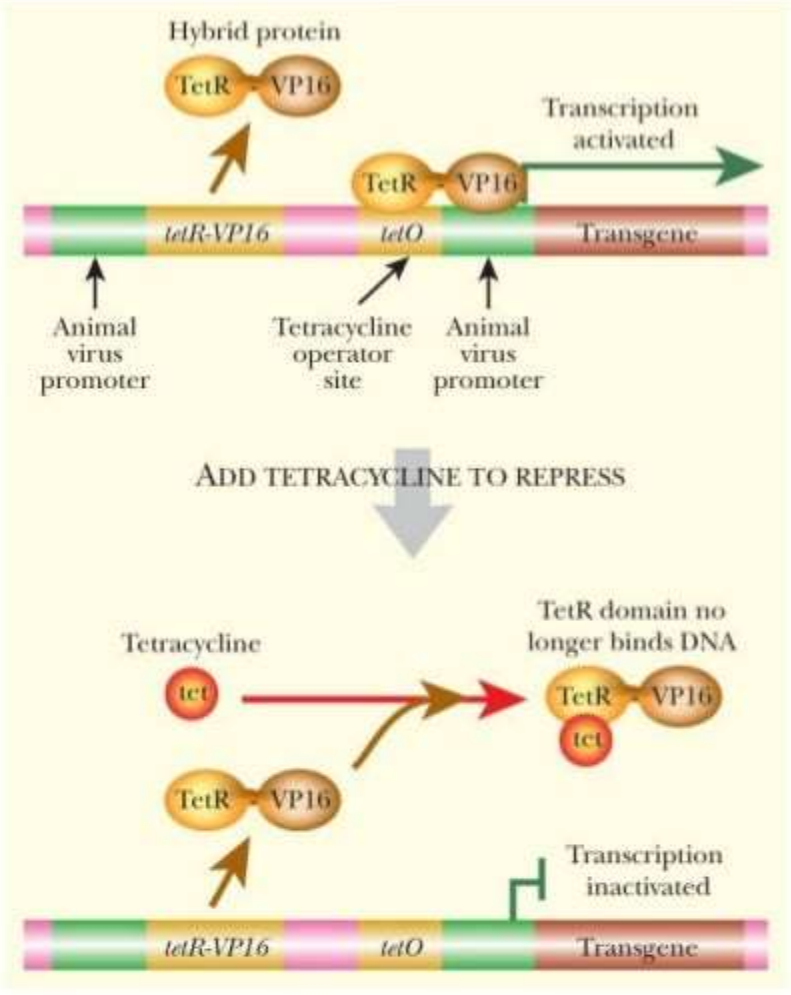

FIGURE 15.11

Hybrid TetR-VP16 Transactivator Systems
A hybrid protein made from TetR and VP16 controls transcription of a transgene. The hybrid protein binds to a TetR operator site, and the VP16 domain activates transcription. When tetracycline is added, the TetR domain binds this and is released from the tetO site. Therefore, tetracycline inhibits transgene expression.

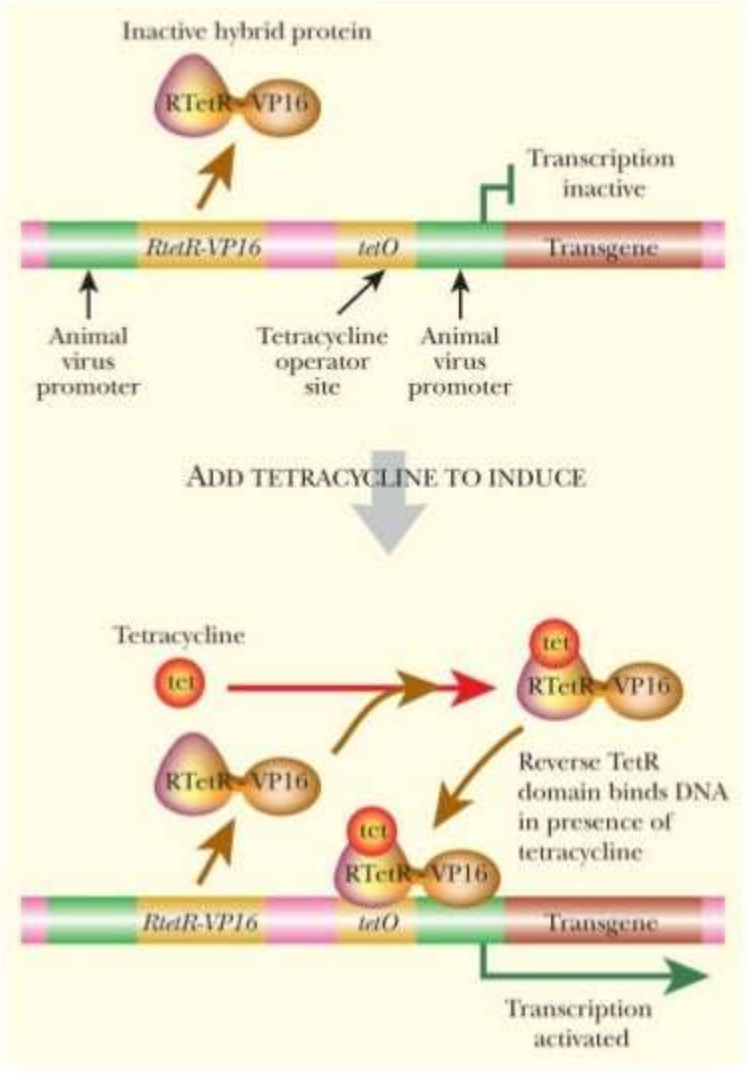

FIGURE 15.12

Reverse TetR-VP16 Transactivator System
The reverse TetR-VP16 hybrid protein only binds to tetO when tetracycline is present. So, in contrast to the previous case (see Fig. 15.11), the transgene is expressed only in the presence of tetracycline.

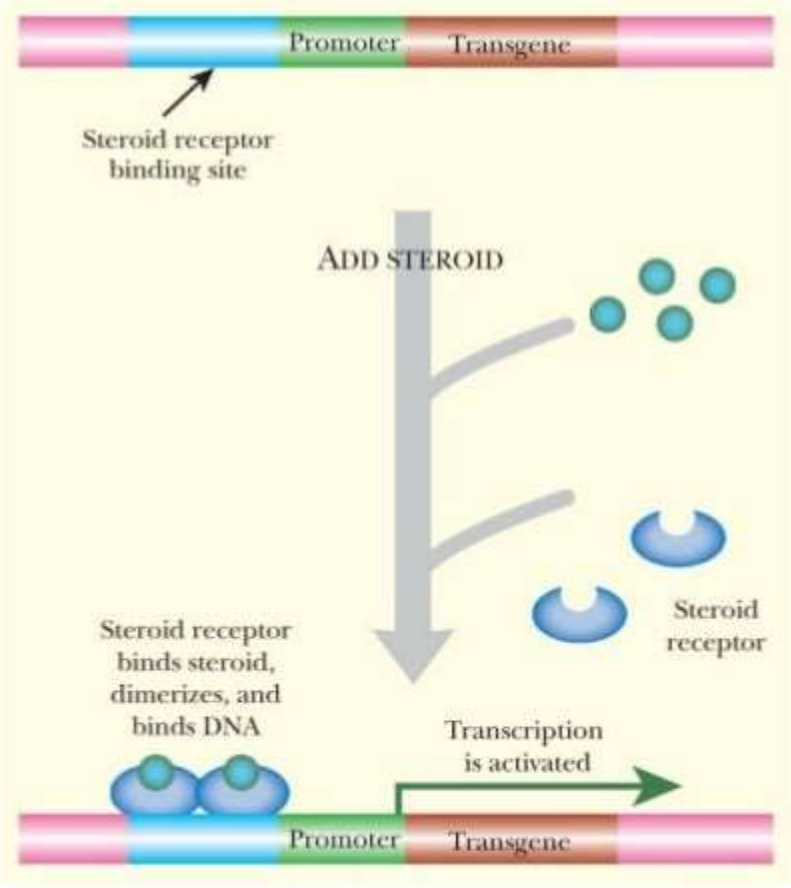

FIGURE 15.13

Steroid Hormone to Activate Transgene
Some transgenic animals have the transgene under the control of a steroid-controlled promoter. When the animal is treated with the steroid, the steroid diffuses across the cell membrane and binds to its receptor in the cytoplasm. The receptor-steroid complex then enters the nucleus, where it binds to the transgene promoter and turns on transcription.

Control by Site-Specific Recombination

- DNA rearrangements that turn transgenes on/off are promoted by either Cre or Flp recombinases
  - Segments are removed or inverted to cause activation
  - Manipulated after successful incorporation into the germline chromosome of host

- Recognition of short specific sequences by DNA binding proteins
  - Between two of the recognition sequences
  - Cre recombinase from bacterial virus P1
  - Flp recombinase from the 2-micron plasmid of yeast
  - Both recognize *loxP* and FRT

- Removal of unwanted segments of DNA requires flanking
  1. Removal of selective markers
  2. Activation of transgene
  3. *In vivo* chromosome engineering
  4. Creation of conditional knockout mutants

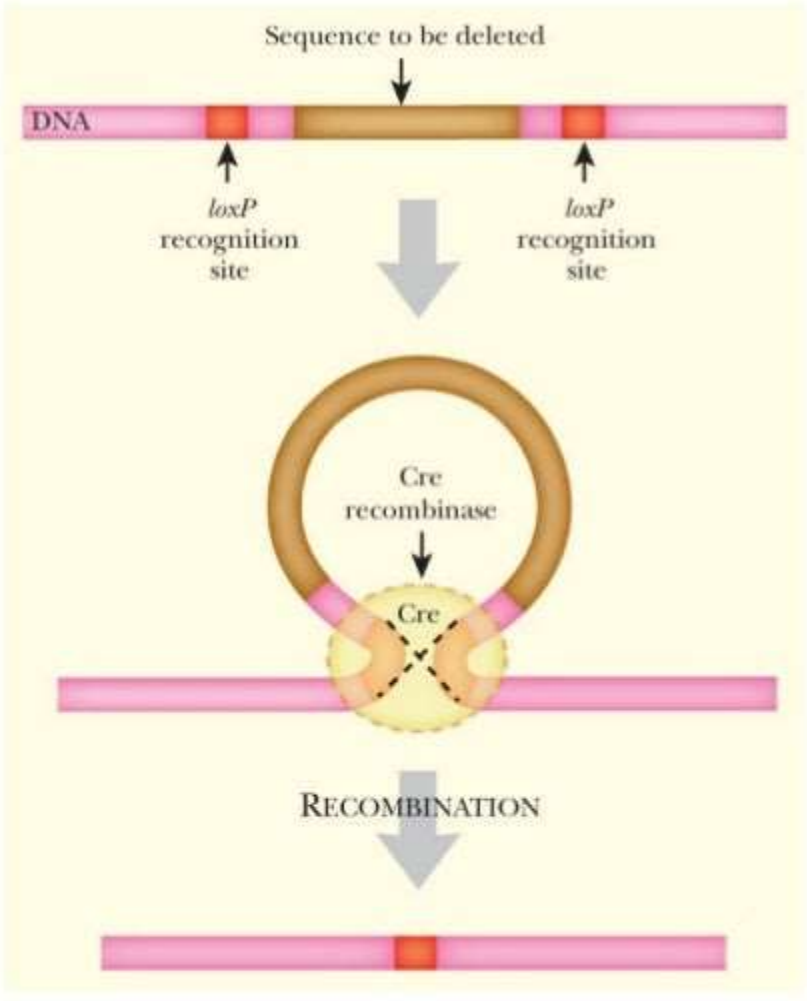

FIGURE 15.14

Site-Specific Deletions in Transgenic Animals
Two loxP sites must be inserted into the DNA, flanking the sequence to be deleted. When Cre recombinase is activated, it recombines the two loxP sites and deletes the segment of DNA between them.

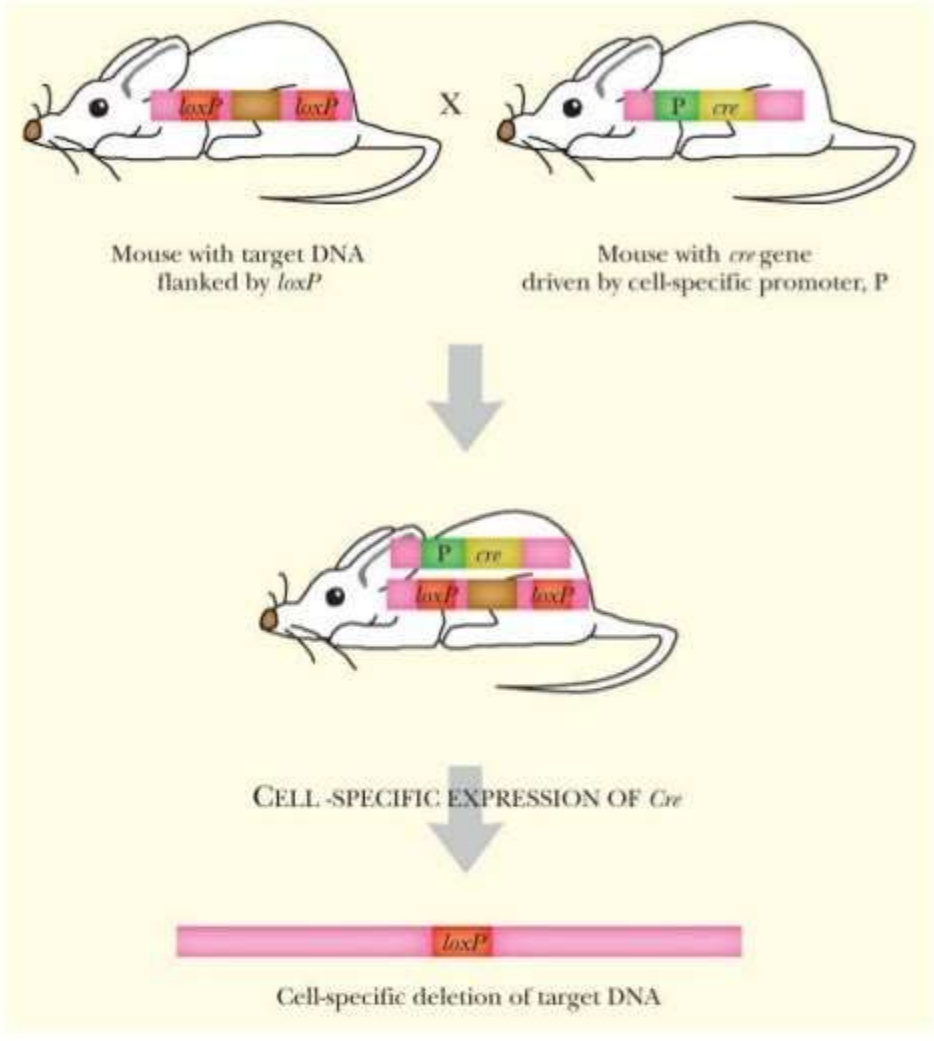

FIGURE 15.15

Two-Mouse Cre/Lox System
One mouse has the target DNA that is to be deleted flanked by two loxP sites. A second mouse has the gene for Cre recombinase under the control of a tissue-specific or inducible promoter. When these two mice mate, some of the offspring will receive a copy of both genetic constructs. When the Cre protein is induced, it will direct the deletion of the target DNA.

Transgenic Insects

- P element transposons are used in *Drosophila* and other insects
  - To introduce transgenic DNA into insects
  - Cause hybrid dysgenesis (high mutation rate that lowers the proportion of viable offspring)
  - Can be used to introduce any sequence of DNA into a strain of susceptible insects
  - Carried on a bacterial plasmid constructed in a bacterial host
  - P element presence monitored by appropriate marker genes

- DNA may be microinjected into embryos of P-negative strains of *Drosophila*
  - Diploid nucleus divides multiple times without cell division creating a syncytium
  - Nuclei migrate to outer membrane where cleavage furrow forms
  - Center remains undivided and provides nutrients to developing larva

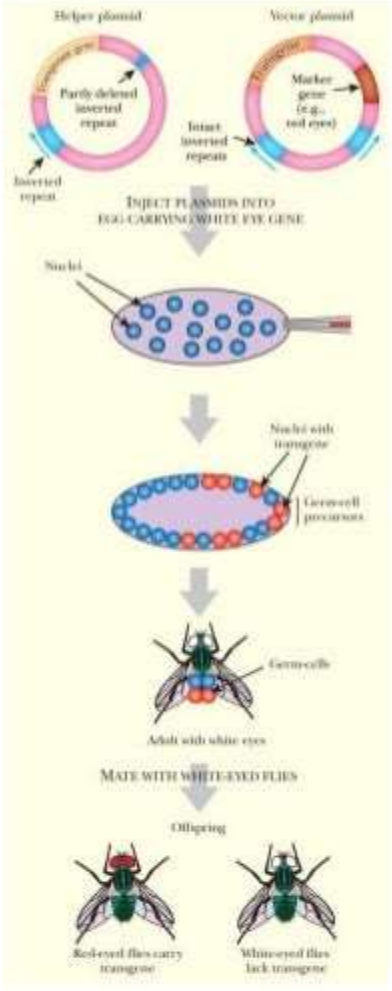

FIGURE 15.16

P Element Engineering in Drosophila

Two different plasmids are used to insert transgenes into Drosophila. The helper supplies the transposase. It carries an immobile P element with one of the inverted repeats deleted but with functional transposase. The second plasmid has the transgene plus marker (a gene for red eyes) flanked by the P element inverted repeats. Both vectors are injected into the posterior end of an egg, which has 2000 to 4000 nuclei within one membrane. The transposase is expressed, which results in random transposition of the transgene (plus marker) into various chromosomes in different nuclei. Hopefully, some insertions occur in germ cell nuclei. The egg is then allowed to form an adult (with white eyes in this case). This fly is then mated to another white-eyed adult. If insertion into the germline was successful, some offspring will express the marker gene and have red eyes.

Genetically Modified Mosquitoes

- DNA inserted into germline cells
  - *piggy*Bac transposon from the cabbage looper
  - *Minos* transposon from *Drosophila hydei*
- Genetically engineering mosquitoes that are resistant to disease agent colonization
  - Noncarrier mosquitos released into the wild to displace disease-transmitting population
- Increase expression of defensin A protein
  - Proteins from other species expressed in mosquito midgut to block transmission
- Genetically engineered human antibodies
  - Artificial genes for single-chain antibodies to circumsporozoite protein of malaria

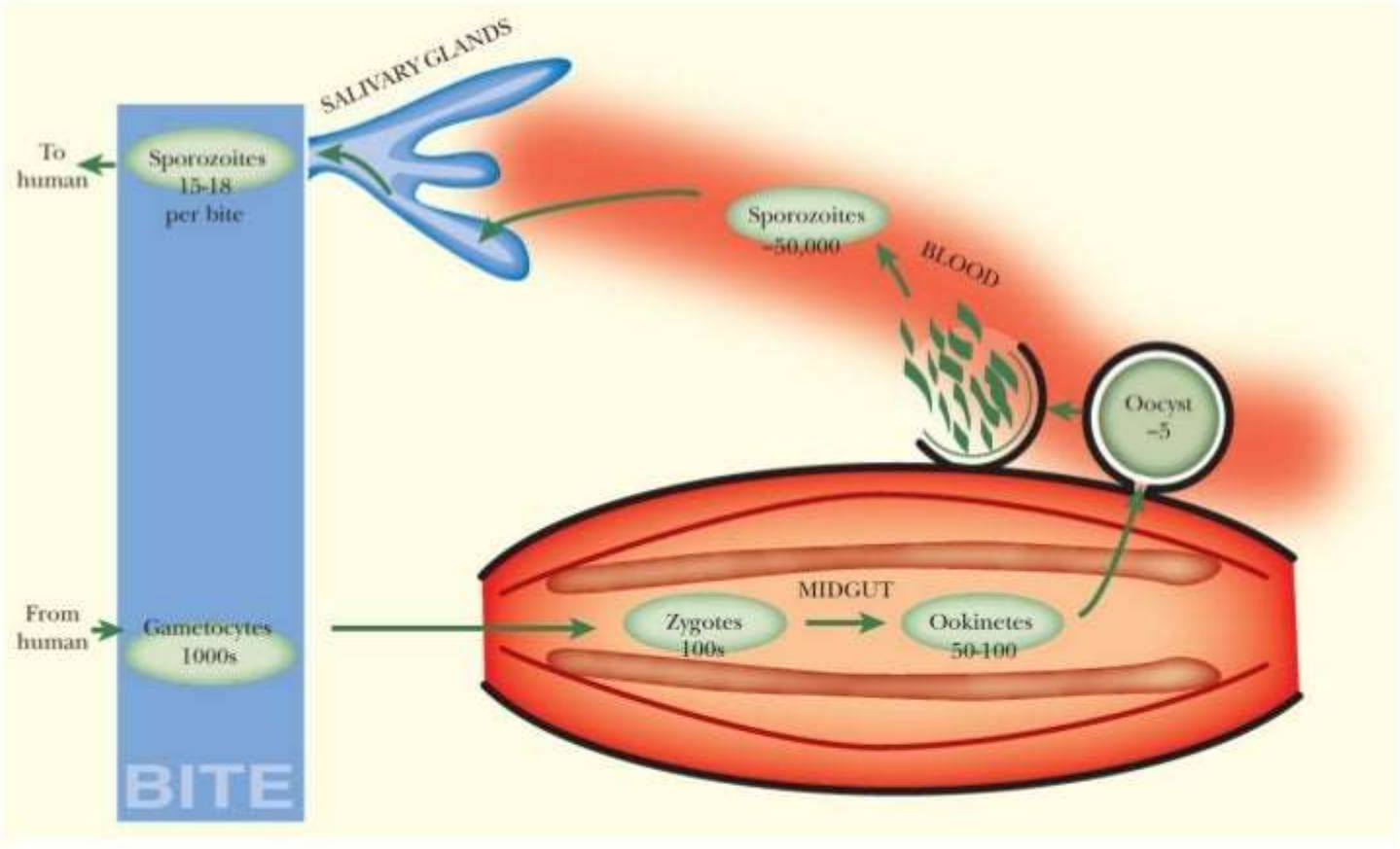

FIGURE 15.17

Development of Plasmodium in the Mosquito
When a mosquito bites a human with malaria, the human blood has thousands of Plasmodium gametocytes (bottom of figure). These travel into the midgut of the mosquito, where they produce hundreds of zygotes. About 50 to 100 of these develop into mobile ookinetes, which then migrate into the hemolymph or blood and give rise to about five oocysts. Each oocyst releases about 50,000 sporozoites that migrate to the mosquito salivary gland. When the mosquito bites another human, only about 15 to 18 sporozoites enter the bloodstream. Plasmodium life stages are shown in green ovals, and mosquito structures are labeled in uppercase.

Cloning Animals by Nuclear Transplantation

- The nucleus from a somatic cell is inserted into an egg cell whose own nucleus has been removed
  - The cytoplasmic environment in the egg cell can reprogram nuclei from somatic cells
- Generates a group of identical cloned animals
  - Several nuclei from the same donor are transplanted into a series of enucleated eggs
  - Fusing somatic cell with an empty egg cell transfers the donor nucleus into a nondifferentiated cytoplasm
  - Electrical pulse fuses the two cell membranes into one embryo
- Dolly
  - First mammal to be produced using a nucleus from a differentiated adult cell line

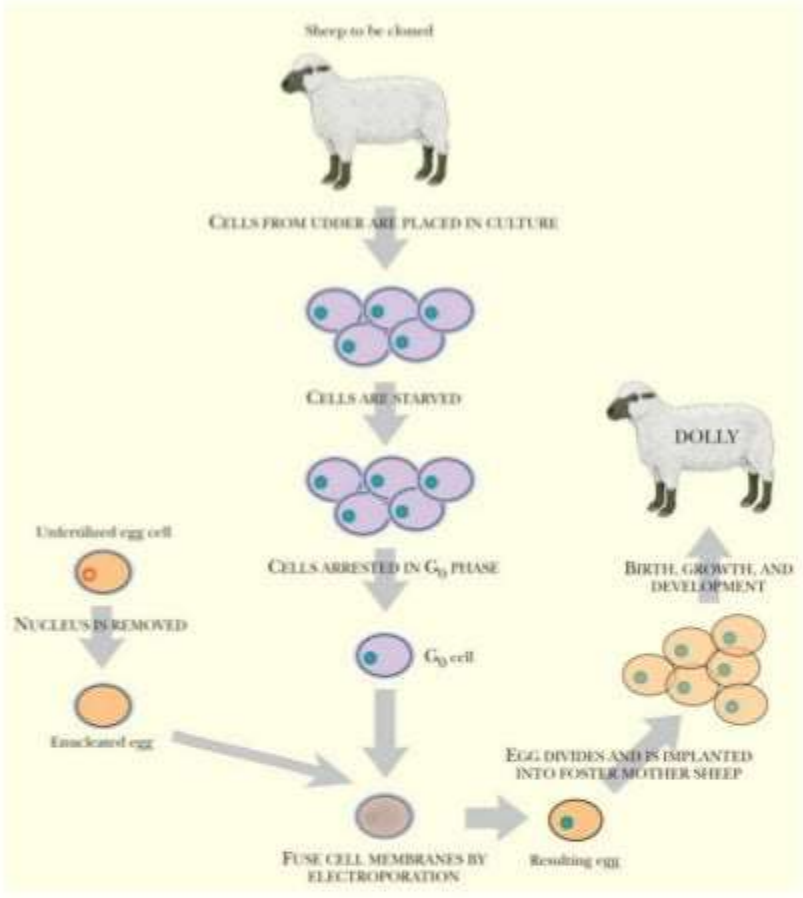

FIGURE 15.18

Scheme for Cloning Sheep
To clone a mammal such as a sheep, cells from the udder are isolated, grown in culture, and then starved in order to arrest them in G0 of the cell cycle. Unfertilized egg cells from another sheep are also harvested and the nucleus is removed from them. An electrical stimulus fuses the G0 udder cell with the enucleated egg, thus placing a somatic cell nucleus into an undifferentiated cytoplasm. The eggs that result are put back into a foster mother, and the offspring are screened for DNA identical to the donor sheep.

Improving Livestock by Pathway Engineering

- Combining pathway engineering with cloning to improve livestock
  - Cysteine biosynthetic pathway absent in mammals
  - Cannot make sulfur-containing amino acids; require dietary supplementation
  - Uptake and degradation by microorganisms found in intestine

- Bacteria synthesize cysteine using two steps
  - Serine plus inorganic sulfide
  - Serine transacetylase and acetylserine sulfhydrylase are encoded by *cysE* and *cysK* genes
  - Cloned from *Salmonella* and under control of the mouse metallothionein promoter
  - Successfully integrated into transgenic mice

- Synthesis of other essential amino acids
  - Lysine and threonine

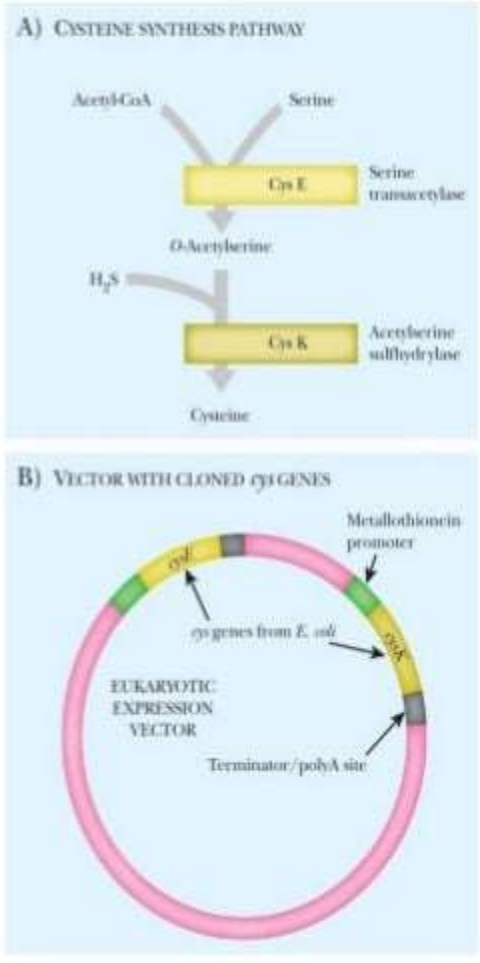

FIGURE 15.19

Biosynthesis of Cysteine from Serine plus Sulfide
(A) The cysteine biosynthetic pathway uses serine and acetyl-CoA. First, acetyl-CoA and serine are converted to O-acetylserine by serine transacetylase. Then acetylserine sulfhydrylase converts O-acetylserine to cysteine using hydrogen sulfide (H2S). (B) The cysteine biosynthetic pathway has been cloned into a eukaryotic expression vector. The two E. coli genes are cloned behind the mammalian metallothionein promoter.

Transgenic People, Primates, and Pets

- Mammalian cloning has high failure rate
  - Methylation pattern of DNA
  - Different between natural embryos and those derived from cloning
- Primates have not been successfully cloned
  - Transgenic rhesus monkeys using retrovirus vectors
  - 2000: first named ANDi carrying *gfp* gene
  - Splitting an embryo at eight-cell stage into four genetically identical embryos
  - Artificial twinning not true nuclear transplantation
- Human cloning for tissue transplantation
  - Therapeutic cloning of reprogrammed cells grown in culture
- Human issues relating to nuclear transplantation
  - Cloning of a human individual is not ethical
  - Fairly risky, financial costs, and production efforts
  - Chance of successful implantation into a surrogate human mother is 3-10x lower than sheep

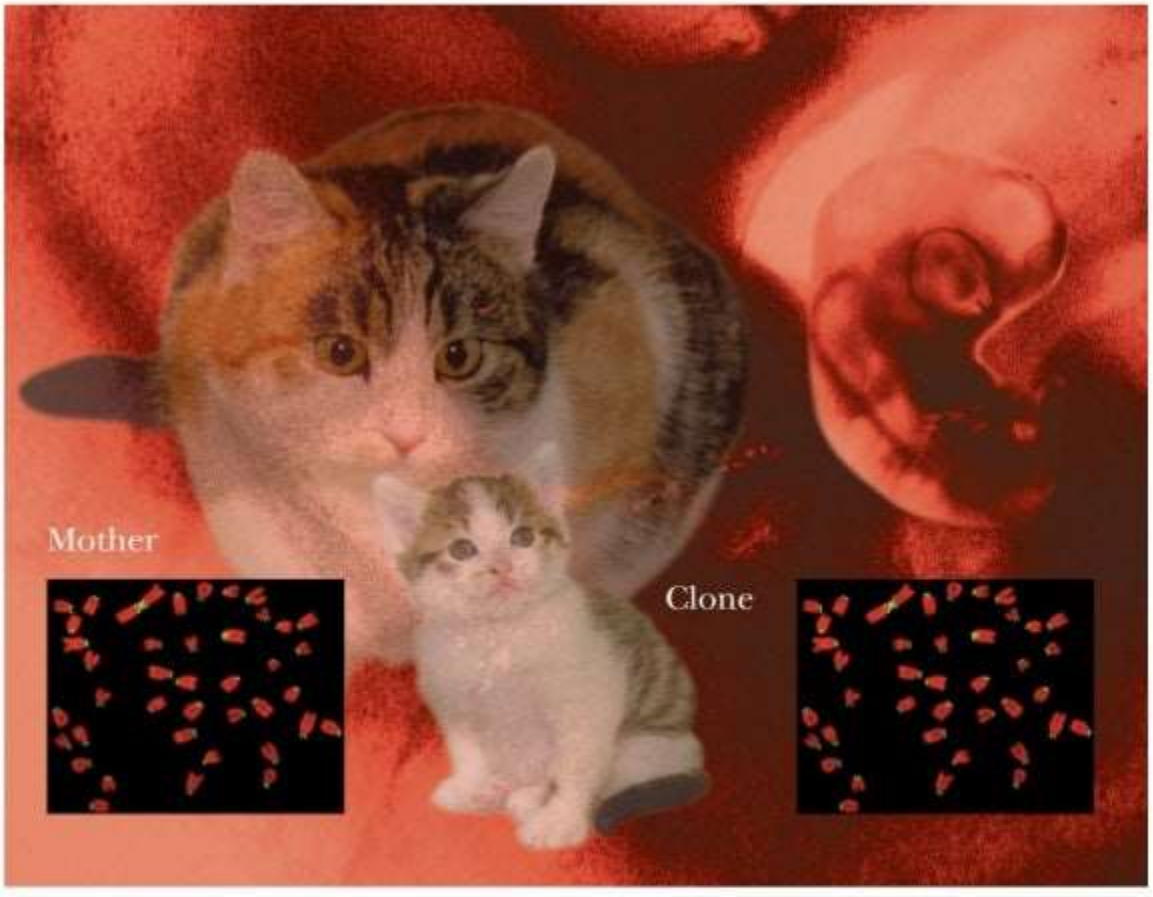

FIGURE 15.20

A Clone Is Not a Copy
This digital collage shows CopyCat and his mother Rainbow. CC is a true clone generated by nuclear transplantation. Rainbow, a calico female is the nuclear donor. Both cats therefore have identical chromosomes. Although CC is a genuine clone, she does not look identical to the nuclear donor because the pattern of a cat's coat is partly due to randomized cell divisions and, in females, X-linked inactivation. Digital media by Hunter O'Reilly, 2002.

RNA Interference

- Manipulation of genes at the RNA level
  - Antisense, ribozymes, and RNA interference
  - Gene expression decreased by inserting genes encoding antisense RNA or encoding ribozymes target to cleave a specific mRNA
- Formation of dsRNA
  - Drop in expression of corresponding gene due to degradation of mRNA
  - Must correspond to the exon sequences of the target gene
- RNA interference triggered by adding RNA directly
  - Or by expression of the corresponding transgenic DNA constructs
  - Neighboring sense and antisense sequences will produce hairpin RNA
  - Single transgene flanked by two promoters; one in each direction
- Homologous cosuppression
  - Multiple copies of transgene decreases expression of related host genes
  - Single copy to endogenous host gene may be sufficient

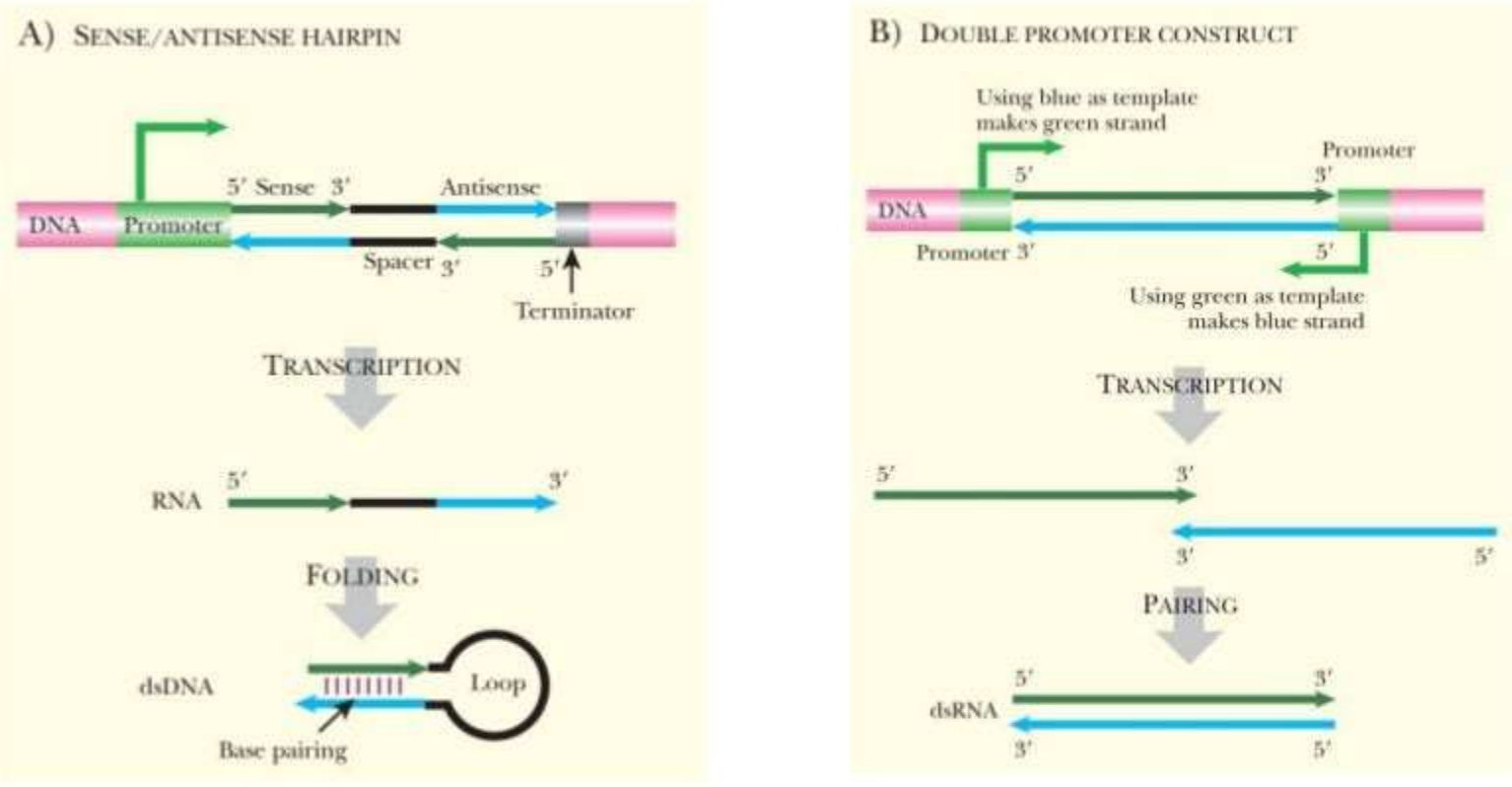

FIGURE 15.21

RNA Interference Constructs
RNA interference occurs when both the sense and antisense RNA of a gene are present and form dsRNA. Two constructs are shown that direct the synthesis of a dsRNA molecule. The first construct (A) has both sense and antisense regions that base pair. A spacer separates the sense and antisense regions and forms a loop at the end of the hairpin. The double promoter construct (B) has two promoters—one for the sense strand and the other for the antisense strand. The two resulting RNA molecules are complementary and form a dsRNA molecule. The presence of dsRNA triggers degradation of mRNA from the corresponding gene.

Natural Transgenics and DNA Ingestion

- Human genome contains genes of foreign origin by lateral/horizontal transfer
  - Bacterial genes
  - Genes originally from higher organisms
  - Transmitted by retroviruses
  - "natural transgenesis"

- Two major pathways for naturally acquisition
  1. Viral transduction
  2. Direct intake of DNA

- Short fragments of DNA from diet can be taken up and incorporated into host chromosomes
  - Up to 1000 bp
  - Rubisco gene sequences found in bloodstream and cell nuclei of various tissues
  - Food-borne DNA capable of infiltrating genome and being expressed unknown

Recent Developments

- Transgenic animal models of neurodegeneration based on human genetic studies

- **Abstract** The identification of genes linked to neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and Parkinson's disease (PD) has led to the development of animal models for studying mechanism and evaluating potential therapies. None of the transgenic models developed based on disease-associated genes have been able to fully recapitulate the behavioral and pathological features of the corresponding disease. However, there has been enormous progress made in identifying potential therapeutic targets and understanding some of the common mechanisms of neurodegeneration. In this review, we will discuss transgenic animal models for AD, ALS, HD and PD that are based on human genetic studies. All of the diseases discussed have active or complete clinical trials for experimental treatments that benefited from transgenic models of the disease.

Fig. 1 Generation of transgenic mouse models based on inheritance patterns of human disease.

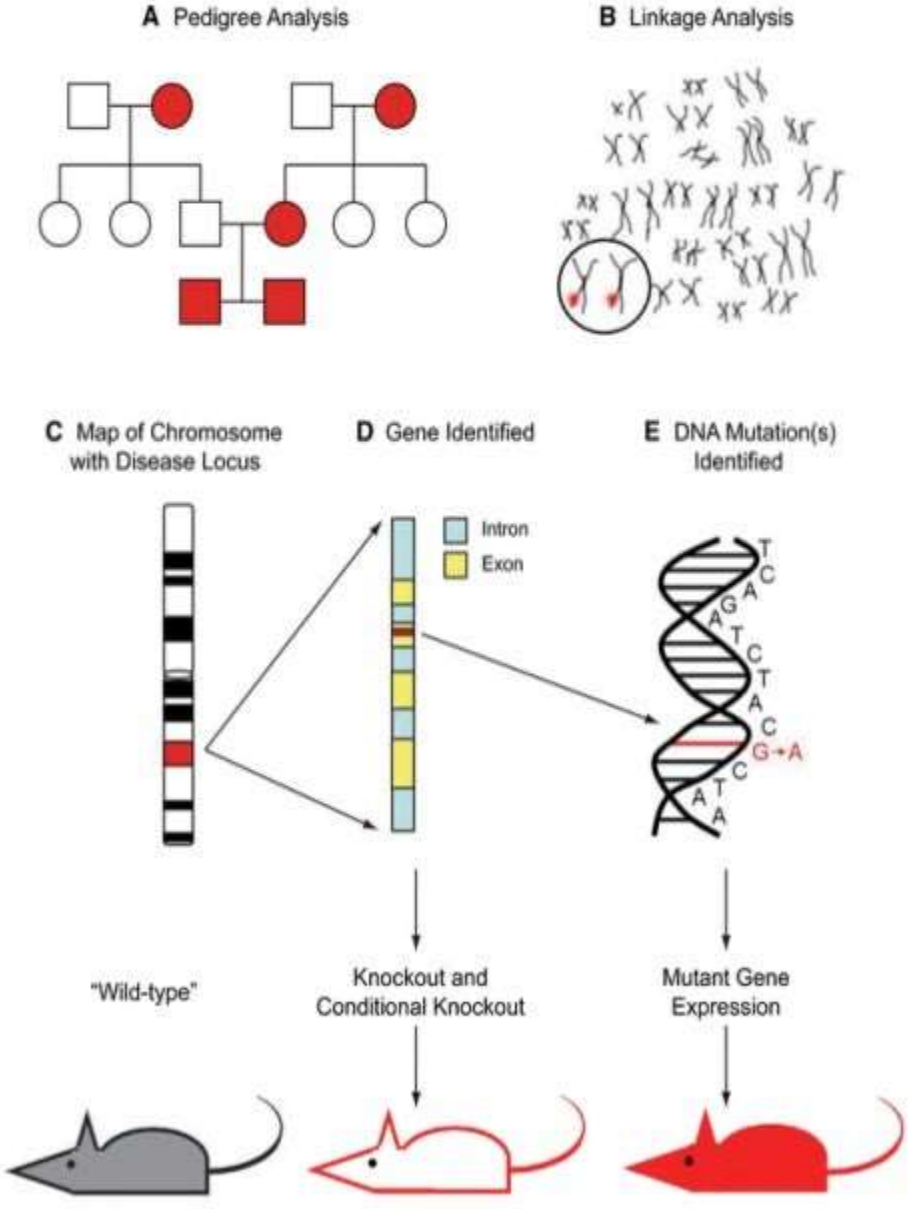

Fig. 1 Generation of transgenic mouse models based on inheritance patterns of human disease. Families or populations with traceable lineages of a disease are subjected to pedigree analysis (**a**) and individuals are subjected to gene linkage studies to identify associated chromosomes (**b**). Further molecular analyses link genetic markers to specific region of chromosome, or locus (**c**) and potentially identify a disease-related gene (**d**). Sequence analysis of putative gene(s) from diseased and non-diseased members of the pedigree can reveal inherited mutation(s) in the gene (**e**). Once the gene and mutation have been identified in affected pedigree members, the mouse ortholog of the human gene can be knocked out completely or conditionally (**d** *arrows*). Also, the mutated human gene can be introduced into the mouse as a replacement for, or in addition to the mouse ortholog (**e** *arrows*). Transgenic mice generated based on the identified gene and its mutants are an important tool when studying behavioral and pathological phenocopy of human disease and when evaluating potential therapeutics for disease

Table 1 Neurodegenerative diseases: symptoms, pathology and associated genetic linkage

Disease	Symptoms	Pathology	Gene map locus	Locus name	Associated gene
Alzheimer's disease (AD)	Dementia	Amyloid-containing plaques in cortex	21q21	AD1	Amyloid precursor protein
	Apraxia	Neurofibrillary tangles	19q13.2	AD2	Apolipoprotein E (APOE)
	Aphasia		14q24.3	AD3	Presenilin-1 (PSEN1)
	Depression, anxiety and delusions		1q31–q42	AD4	Presenilin-2 (PSEN2)
	Short attention span		12p11.23–q13.12	AD5	Not yet determined
Amyotrophic lateral sclerosis (ALS)	Muscle weakness and wasting	Loss of lower motor neurons in ventral horn of spinal cord	21q22.1	ALS1	Superoxide dismutase-1 (SOD1)
	Muscle fasciculations and cramping	Degeneration of corticospinal tracts	2q33	ALS2	Alsin
	Impaired limb dexterity	Neurodegeneration in primary motor cortex	18q21	ALS3	Not yet determined
	Respiratory failure	Reactive astrocytes in motor cortex and spinal cord	9q34	ALS4	Senataxin (SETX)

Recent Developments

- Transgenic pigs as models for translational biomedical research

Abstract The translation of novel discoveries from basic research to clinical application is a long, often inefficient, and thus costly process. Accordingly, the process of drug development requires optimization both for economic and for ethical reasons, in order to provide patients with appropriate treatments in a reasonable time frame. Consequently, "*Translational Medicine*" became a top priority in national and international roadmaps of human health research. Appropriate animal models for the evaluation of efficacy and safety of new drugs or therapeutic concepts are critical for the success of translational research. In this context rodent models are most widely used. At present, transgenic pigs are increasingly being established as large animal models for selected human diseases. The first pig whole genome sequence and many other genomic resources will be available in the near future. Importantly, efficient and precise techniques for the genetic modification of pigs have been established, facilitating the generation of tailored disease models. This article provides an overview of the current techniques for genetic modification of pigs and the transgenic pig models established for neurodegenerative diseases, cardiovascular diseases, cystic fibrosis, and diabetes mellitus.

Figure 1: Current techniques

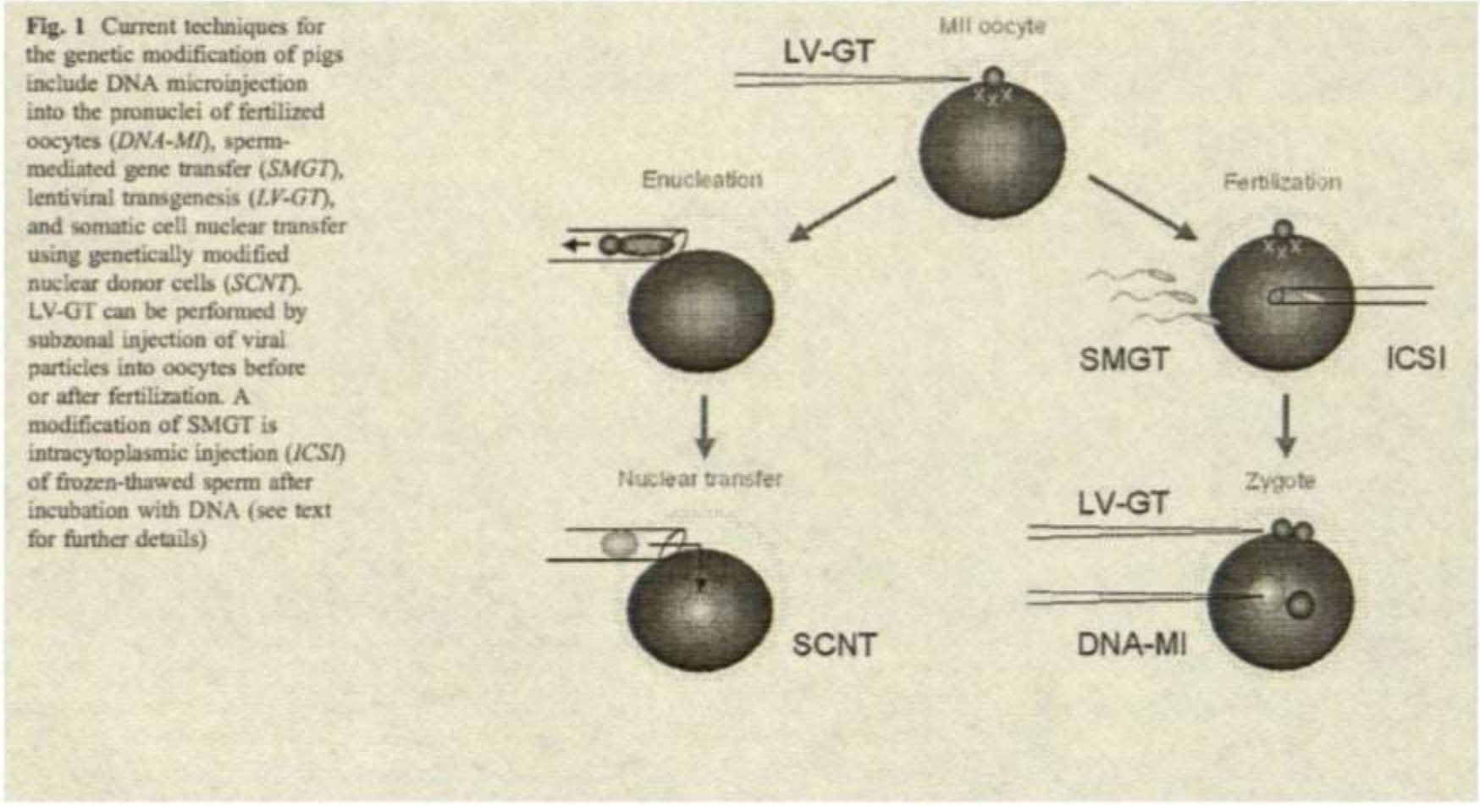

Fig. 1 Current techniques for the genetic modification of pigs include DNA microinjection into the pronuclei of fertilized oocytes (*DNA-MI*), sperm-mediated gene transfer (*SMGT*), lentiviral transgenesis (*LV-GT*), and somatic cell nuclear transfer using genetically modified nuclear donor cells (*SCNT*). LV-GT can be performed by subzonal injection of viral particles into oocytes before or after fertilization. A modification of SMGT is intracytoplasmic injection (*ICSI*) of frozen-thawed sperm after incubation with DNA (see text for further details)

Figure 2: Somatic Cell Nuclear Transfer

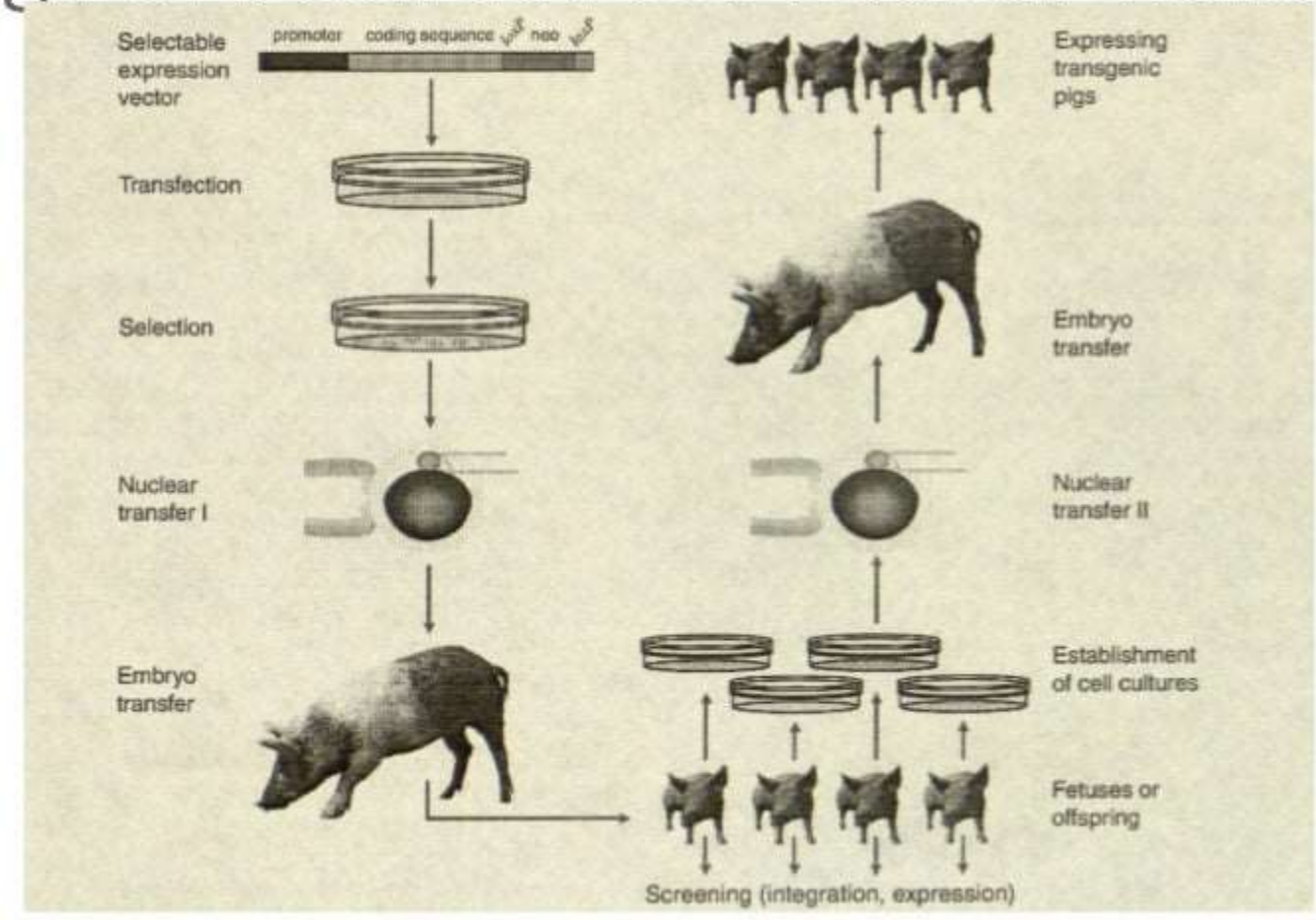

Fig. 2 Efficient production of transgenic pigs by using somatic cell nuclear transfer. An expression vector carrying a removable selection cassette is transfected into nuclear donor cells. After selection, the resulting transgenic cells are pooled and used for nuclear transfer. Pooling of cell colonies reduces the time in culture and allows the generation of independent founder fetuses/offspring in one litter. Cloned embryos are transferred to synchronized recipients. Depending on the expected onset and tissue specificity of transgene expression, pregnancies may be terminated to recover fetuses, or birth and early development of offspring is awaited. Fetuses or tissues from born offspring are processed for transgene integration and expression studies, while individual cell cultures are established for re-cloning of the fetuses/offspring with the most suitable integration/expression pattern

Figure 4: Advanced Preclinical Animal Models

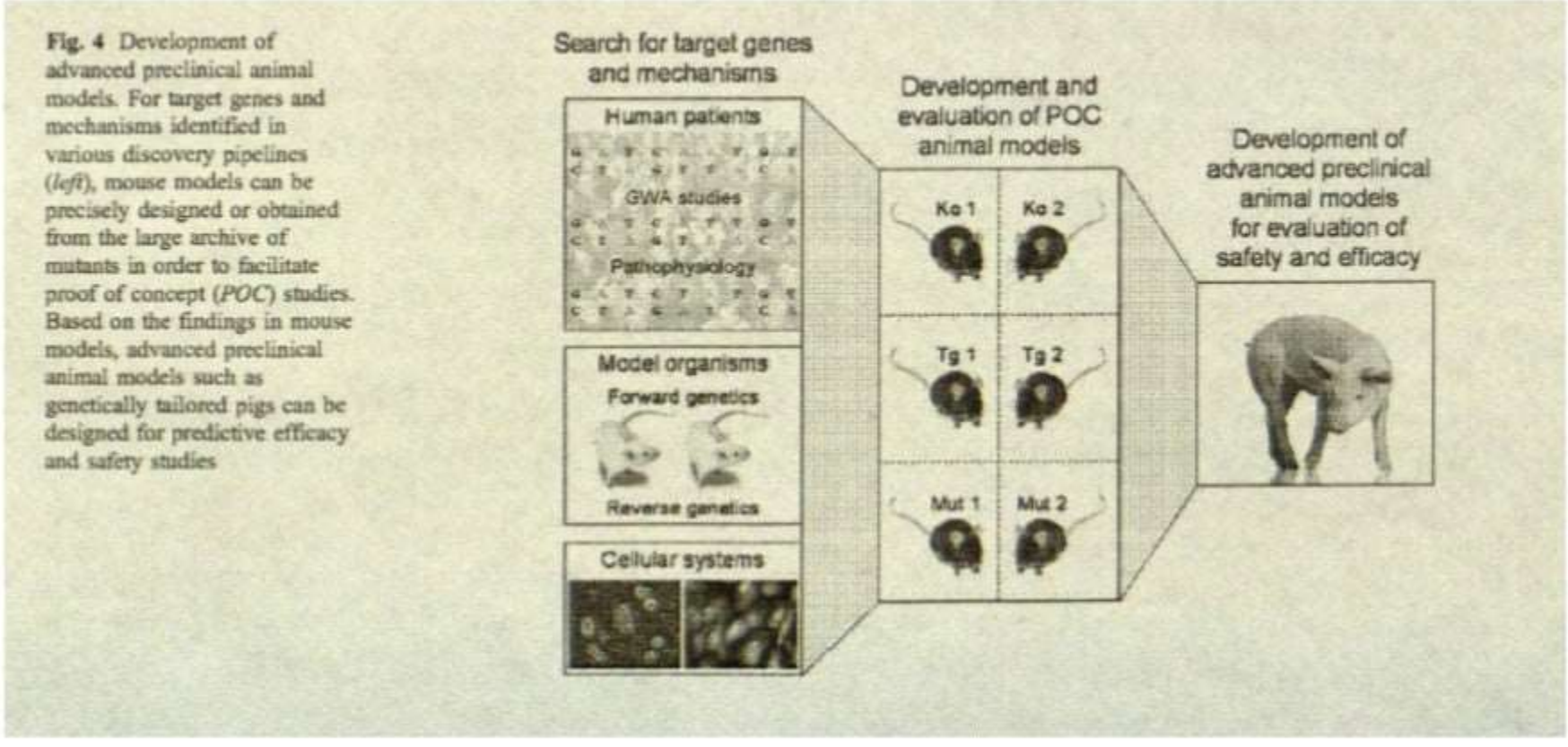

Fig. 4 Development of advanced preclinical animal models. For target genes and mechanisms identified in various discovery pipelines (*left*), mouse models can be precisely designed or obtained from the large archive of mutants in order to facilitate proof of concept (*POC*) studies. Based on the findings in mouse models, advanced preclinical animal models such as genetically tailored pigs can be designed for predictive efficacy and safety studies