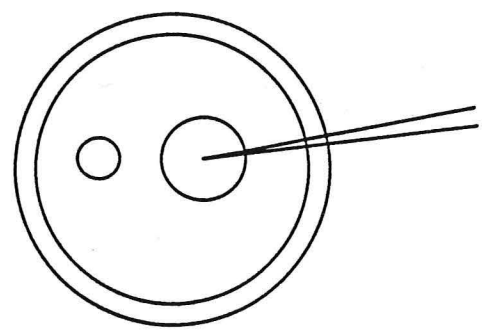
Internal Medicine Grand Rounds

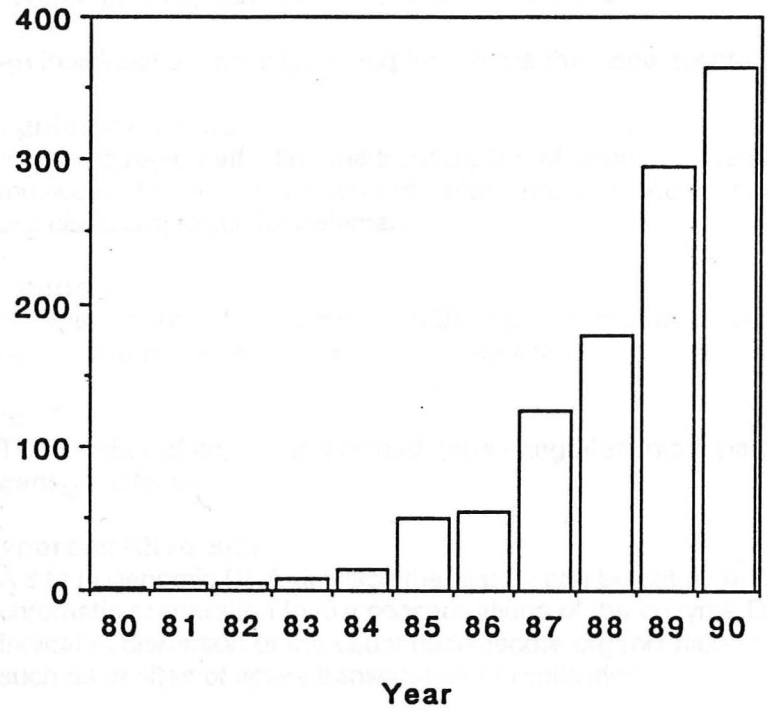
Transgenic Animals: Application to the Study of Human Disease



Joel D. Taurog, M.D.

May 9, 1991

Figure 1. Yearly Output of Publications on Transgenic Mice, 1980 -1990



Source: BRS Colleague Medline

Table I. Definitions Pertinent to Transgenic Technology

Blastocyst Embryo at the time of implantation into the uterine wall. Can accept injection of pluripotent embryonic stem cells.

cDNA DNA complementary to an RNA molecule, synthesized from the RNA by reverse transcription in vitro. Contains no introns.

Chimera An individual animal developing from more than one zygote.

***cis*-acting regulatory element**
A genetic region affecting the transcription of genes on the same DNA molecule. Promoters, enhancers, silencers, and locus control regions are *cis*-acting regulatory elements.

Constitutive gene
A gene expressed as a function of the interaction of its promoter with RNA polymerase, without additional regulation.

Copy number
The number of copies of a cloned gene integrated into a particular transgene locus.

DNAase I hypersensitive site
A site in genomic DNA at which the duplex can be cut by exposing a chromatin preparation to low concentrations of the enzyme DNAase I. Indicates disruption of the usual nucleosome organization of chromatin, such as at sites of active transcription or replication.

Embryonic stem cell
Cells from an embryo that can give rise to all of the tissues of the adult animal. Can be grown in culture as a stable line, susceptible to selective agents, thus providing the basis for gene targeting.

Enhancer A *cis*-acting transcriptional regulatory sequence that increases the utilization of a specific promoter. Unlike a promoter, it functions in either orientation and in any location relative to the promoter. Often tissue-specific.

Exon Any segment of an interrupted gene that is represented in the mature RNA product.

Founder An animal resulting from an embryo into which DNA has been introduced that is potentially capable of transmitting a stably integrated transgene to its offspring. Gives rise to a transgenic line.

Gene targeting
Directing the site of integration of a transgene, as opposed to relying upon random integration. Based on the phenomenon of homologous recombination.

Genomic DNA
DNA with a sequence as it occurs naturally in vivo.

Germ line Pertaining to the cells from which gametes are derived. A transgene that is not integrated into at least some germ line cells will not be transmitted to the next generation.

Hemizygous
The usual state of a transgene locus. Indicates the presence of the locus on one member of a pair of chromosomes.

Homologous recombination
Recombination within a cell between two segments of double-stranded DNA on the basis of identical nucleotide sequence. Provides a method for targeting the site of insertion of a transgene by including within the construct sequences that are identical to sequences in the host genome.

Homozygous
Having the same transgene locus on each of a pair of chromosomes. A state attained by breeding hemizygous animals of the same transgenic line.

Insertional mutation
A mutation that has resulted from integration of a transgene at a site that alters the function of an endogenous gene. May be dominant or recessive; often lethal.

Intron A segment of DNA that is transcribed into RNA, but then subsequently removed from within the RNA transcript by splicing together the exons on either side of it. Not present in the mature RNA transcript.

Locus control region
A *cis*-acting regulatory region containing DNAase I-hypersensitive sites that confers position-independent expression of genes to which it is linked (i.e., expression in transgenic animals is proportional to copy number and independent of the site of integration). First identified in the ß-globin gene cluster.

Mosaic An animal arising from a single zygote but composed of cell lineages with at least two nonidentical genomes. Results from integration of a transgene locus after DNA replication has already occurred within the embryo.

Promoter A region of DNA involved in binding RNA polymerase to initiate transcription. For most structural genes, the promoter region consists of DNA sequences located near the transcriptional start site.

Pronucleus
Seperate nucleus of the male or female gamete in a fertilized egg before fusion.

Pseudopregnant female
Female that has been bred during natural estrus with a vasectomized male, and therefore physiologically adapted for pregnancy. Used as recipient for fertilized eggs microinjected with DNA.

Superovulation
The experimental induction of the release of an abnormally large number of ova from a female by homonal manipulation.

***trans*-acting regulatory element**
A factor, usually a protein, that influences the transcription of a gene other than the one that encodes it. Usually acts by binding to sequences of *cis*-acting regulatory sequences, to another *trans*-acting factor, or to RNA polymerase.

Transfection
Incorporation of added DNA by eukaryotic cells in vitro.

Transgenic animal
An animal bearing a gene integrated into its genome as a result of experimental manipulation of the embryo that gave rise either to the animal itself or to an ancestor of the animal.

Table II. Some Important Historical Landmarks in the Development of Transgenic Technology

1949 Culturing mouse 8-cell eggs to blastocyst stage (Hammond)

1953 Structure of DNA identified, eventually leading to recombinant DNA technology (Watson and Crick)

1956 Culturing mouse 2-cell eggs to blastocyst stage (Whitten)

1961 Production of chimeric mice by fusing 8-cell embryos (Tarkowski)

1963 Establishment of microdroplet-under-oil system for culturing mouse 2-cell eggs (Brinster)

1968 Production of chimeric mice by transferring cells from one blastocyst to another (Gardner)

1973 DNA-mediated transfer and expression of viral genes in mammalian cells in vitro (Graham and van der Eb)

1974 Teratocarcinoma cells colonize the mouse blastocyst and appear in the adult (Brinster)

1975 Teratocarcinoma cells colonize the mouse blastocyst and form part of the germ line of the adult (Mintz and Illmensee)

1976 Germ line integration into mouse embryos and Mendelian transmission of the murine retrovirus Moloney leukemia virus (Jaenisch)

1977 Expression of DNA microinjected into frog *(Xenopus)* eggs (Gurdon)

1980 Pronuclear microinjection of recombinant DNA into mouse eggs with incorporation into the recipient genome (Gordon and Ruddle)

1981 Transgene expression and heritable transmission following pronuclear injection of recombinant DNA into mouse eggs (Brinster and Palmiter; others)

1981 Stable lines of pluripotent embryonic stem cells established from normal mouse blastocyst (Evans and Kaufman; Martin)

1982 Production of "giant" mice by pronuclear microinjection of the rat growth hormone gene fused to the metallothionein promoter (Brinster and Palmiter)

1983 Tissue-specific expression of a transgene following pronuclear microinjection - immunoglobulin kappa chain in B cells (Brinster, Storb, et al).

1984 Pluripotent embryonic stem cells introduced into a blastocyst contribute to all tissues of a chimeric mouse, including the germ line (Bradley, et al.)

1985 Transgenic rabbits, sheep, and pigs produced by pronuclear microinjection (Hammer, et al.)

1986 Production of mice with a heritable transgene by blastocyst injection of a transfected embryonic stem cell line (Kemler, et al.)

1987 Homologous recombination of foreign DNA in mouse embryonic stem cells (Capecchi et al; Smithies, et al.)

1989 Germ line transmission in mice of a mutation introduced into embryonic stem cells by homologous recombination (Thompson, et al.; Robertson, et al.)

References 1-9

Table III. Pronuclear Microinjection of Fertilized Eggs

Most widely used technique for producing transgenic animals

Linearized DNA lacking vector sequences, 200 - 500 copies

Integration frequency 0 - 40%

Integration usually precedes DNA replication (30% are mosaic)

Integration usually all at one, probably random, chromosomal site

Integration usually in a tandem, head-to-tail array

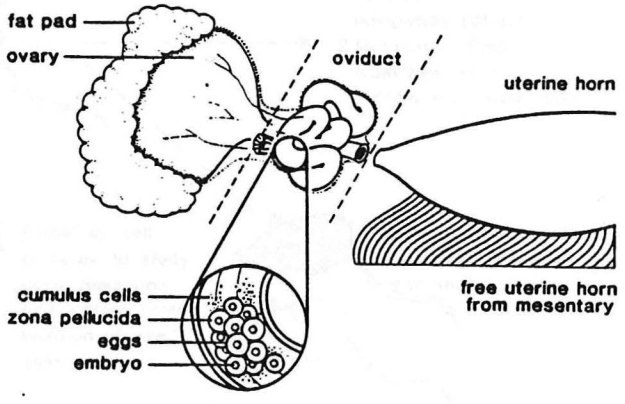
Integration of transgene DNA detected by hybridization

Integrated transgene usually stably transmitted

Expression of transgene detected at mRNA and/or protein levels

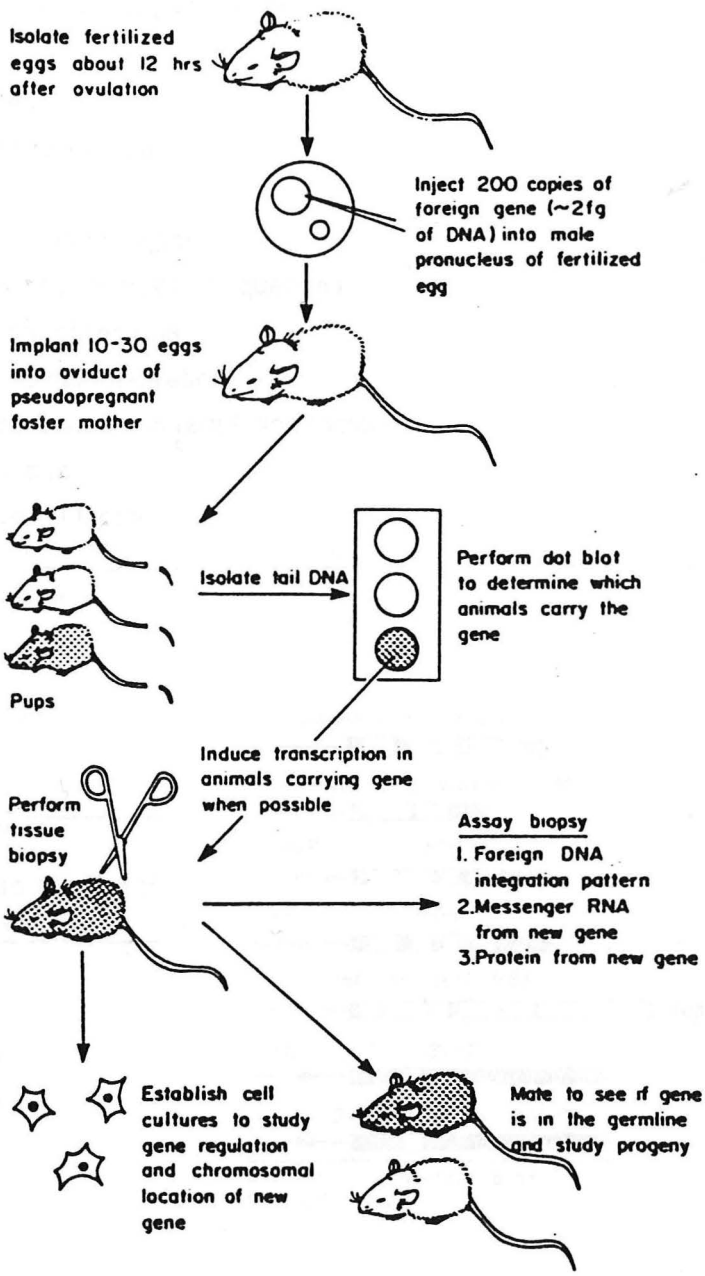
References 2, 3

Figure 2. Isolation of the Mouse Oviduct and Fertilized Eggs



From reference 10

Figure 3. Introduction of Foreign DNA into Mice and Detection of its Expression



From reference 3

Table IV. Factors Influencing Transgene Expression

DNA Constructs

- *cis*-acting regulatory elements
- Genomic clones
- Minigenes
- Hybrid or fusion genes
- Introns
- Polyadenylation region
- Absence of prokaryotic sequences
- Locus control regions

Site of chromosomal integration

Trans-acting transcriptional regulation factors

Host genetic factors

Host environmental factors

References 2, 3, 11

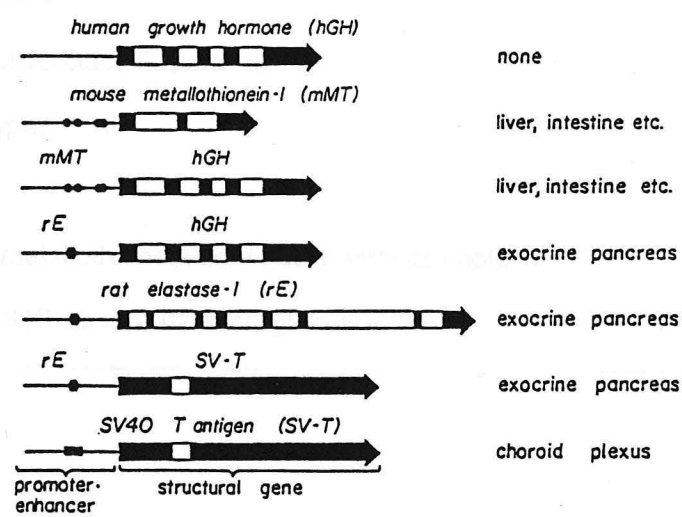


Figure 4.
Fusion Genes Redirect Gene Expression

Reference 3

Table V. Characteristics of Transgene Expression

Developmental Regulation

Transgenes are usually activated at the appropriate time in development
(implies evolutionary conservation of *trans*-acting signals regulating developmental expression)

Tissue Specificity

Dependent primarily upon *cis*-acting regulatory elements

Genes expressed predominantly in one cell type

Examples: immunoglobulins, elastase I, lens crystallin, myelin, insulin, hepatitis B virus

Generally expressed appropriately, if expressed at all (implies evolutionary conservation of *trans*-acting signals for tissue-specific expression)

Levels of expression vary widely among founders

Genes expressed in a few cell types

Examples: α-fetoprotein, MHC class II, transferrin

Genes expressed in many cell types

Examples:

Heterologous promoters: metallothionein constructs, viral promoters

Homologous promoters: MHC class I, ß2-microglobulin

References 2, 3, 5, 7, 11-13

Table VI. Some Examples of Transgenic Models of Human Disease

Viral Constructs

Construct: Hepatitis B virus envelope region/ albumin promoter
Process: Acute and chronic hepatitis

Construct: HBV major envelope polypeptide/ albumin promoter
Process: Hepatocellular carcinoma

Construct: Human poliovirus receptor
Process: Paralyitic poliomyelitis upon inoculation with poliovirus

Neoplasia

Construct: *c-myc*, *Ha-ras*, *neu* with MMTV LTR
Process: Mammary carcinoma

Construct: SV40 large T antigen with tissue-specific promoters
Process: Tissue-specific neoplasia

Construct: *bcr/abl* translocation junction
Process: Acute leukemia

Construct: *int-2* with MMTV LTR
Process: Mammary carcinoma (females); prostatic hyperplasia (males)

Overexpression of normal hormones or cytokines

Construct: Human erythropoietin
Process: Polycythemia

Construct: Interleukin-5 with CD2 promoter
Process: Eosinophilia

Construct: Human apolipoprotein CIII
Process: Hypertriglyceridemia

Expression of disease-related human genes

Construct: Human hemoglobin S (α1-globin and β^s-globin)
Process: Red cell sickling

Construct: HLA-B27 and human ß2-microglobulin
Process: Spondyloarthropathies in rats

Construct: Various genes driven by the rat insulin promoter
Process: Diabetes mellitus (inflammatory or non-inflammatory)

Construct: Defective α1(I) collagen (type I)
Process: Osteogenesis imperfecta

References 14-27

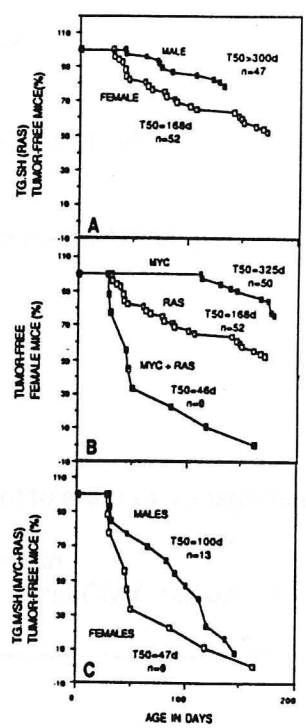


Figure 5. Kinetics of tumor formation in male and female mice bearing *c-myc* and/or activated *Ha-Ras* transgenes under control of the MMTV LTR. The two oncogenes show cooperativity in the rate of tumor formation. From reference 17.

Table VII. Some Examples of Transgenic Models of Insulin-Dependent Diabetes Mellitus

MHC genes under control of rat insulin promoter

Construct: H-2K^b (mouse MHC class I)
Process: Diabetes (non-inflammatory). Reversibly tolerant to transgene product.

Construct: I-A^d (mouse MHC class II)
Process: Diabetes (non-inflammatory). Not tolerant to transgene product.

Construct: I-A^k (mouse MHC class II)
Process: No diabetes. Not tolerant to transgene product.

Non-MHC genes under control of rat insulin promoter

Construct: Interferon-γ
Process: Diabetes (inflammatory). Loss of tolerance to islet cells.

Construct: SV40 large T antigen
Process: Islet cell autoimmunity if expression developmentally delayed.

Construct: Calmodulin
Process: Diabetes (neonatal, non-inflammatory).

Viral genes under control of rat insulin promoter

Construct: Influenza hemagglutinin
Process: Diabetes (inflammatory, low frequency). Not tolerant to transgene product.

Construct: Lymphocytic choriomeningitis virus glycoprotein
Process: Diabetes (inflammatory) following infection with LCMV, associated with T cell immunity to transgene product.

References 28-39

Table VIII. Correction of Naturally Occurring Genetic Disorders of Mice

Construct: MT-rat growth hormone
Process: Correction of GRF receptor deficiency

Construct: Human orinithine transcarbamylase (OTC)
Process: Correction of X-linked OCT deficiency

Construct: Tyrosinase
Process: Correction of albinism

Construct: Myelin basic protein
Process: CNS myelin deficiency

References 40-43

Table IX. Some Examples of Potential Therapeutic Applications of Transgenic Technology

Construct: Multidrug resistance gene (*MDR1*)
Application: Screening MDR antagonists

Construct: Human Factor IX or α1-antitrypsin fused to ovine ß-lactoglobulin
Application: Secretion of functional protein product in milk

Construct: Human immunoglobulin minilocus
Application: Production of antibodies with human constant region domains

Construct: Mouse *Mx1* gene
Application: Intracellular resistance to influenza infection

References 44-48

Table X. Application of Transgenic Technology to the Problem of Immunologic Tolerance

Correctly rearranged T cell receptor or immunoglobulin genes are functionally expressed on the majority of T or B cells

Co-expression of a TCR or Ig and its antigen has several possible outcomes:

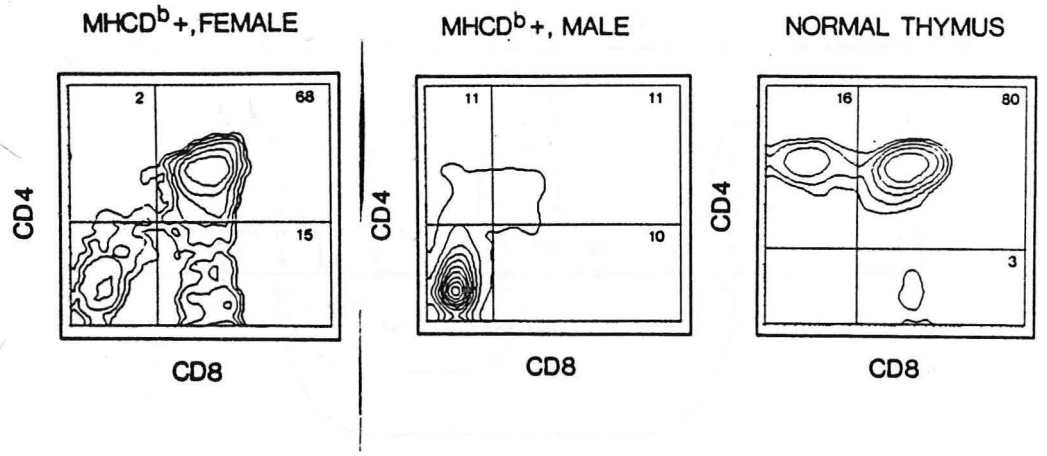
Clonal deletion in the thymus (T cells) or periphery (B cells)

Irreversible inactivation in the periphery (clonal anergy)

Reversible inactivation in the periphery

References 49-57

Figure 6. Positive and Negative Selection of Thymocytes Expressing Transgenic T Cell Receptor α and ß Genes



Effects on thymic selection of a transgenic T cell receptor from a CD8+ T cell clone specific for the male H-Y antigen and the mouse class I antigen H-2D^b. Female D^b transgenic mice, which lack the H-Y antigen, show marked positive selection of CD4-,8+ cells in the thymus. Male D^b transgenic mice, which express the H-Y antigen, show profound depletion of CD4+,8+ and CD4-,8+cells. From reference 57.

Table XI. Application of Transgenic Technology to the Problem of Globin Gene Regulation

Locus control region: 20 kb region 5' to the ß-globin cluster contains conserved DNAase I hypersensitive sites

Confers position-independent expression of ß-globin genes

Controls developmental switching (ε to γ to ß)

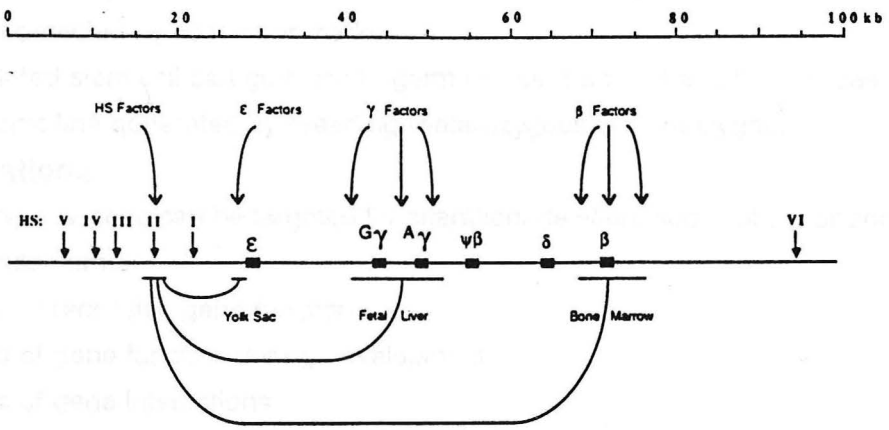
Makes chromatin accessible to trans-acting factors

Similar LCR recently found 5' to the α-globin cluster

May be a general mechanism for developmental regulation of multigene loci

References 58-68

Figure 7. The ß-Globin Locus



The DNAase I hypersensitive sites of the locus control region, 5' of the ε locus, control the developmental switching of globin genes in transgenic mice.

Table XII. Gene Targeting by Homologous Recombination

Homologous Recombination

Requisite enzymatic machinery is intrinsic to cells

Permits introduction of cloned DNA into defined segment of chromosomal DNA

Frequency proportional to length of homologous DNA

Less frequent than nonhomologous (random) integration

Cloned DNA can contain selectable markers

Not dependent upon ends of cloned DNA construct

No apparent positional effect (same frequency of recombination for different constructs)

Embryonic Stem Cells

Can grow in culture and remain pluripotent

Susceptible to selectable markers (examples: neomycin resistance, thymidine kinase)

Become integrated into developing embryos after injection into blastocyst

Animals

Injected blastocyst develops into chimeric animal

Lineage detected by coat color marker

Manipulated stem cell can give rise to germ line cells as well as other tissues

Transgenic line generated by breeding (heterozygous or homozygous)

Applications

In theory, any gene can be targeted for alteration, deletion, substitution, or addition.

This would permit:

Analysis of recessive gene function

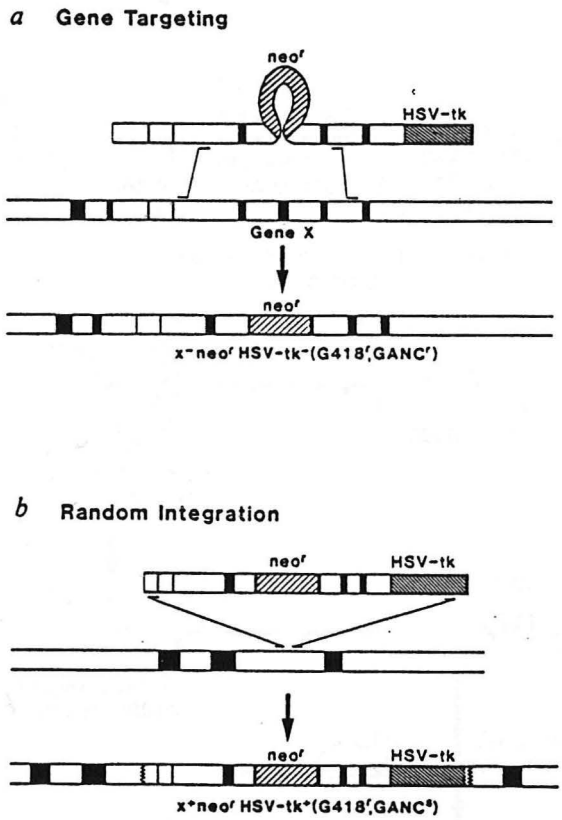
Analysis of gene function during development

Analysis of gene interactions

Production of animal models of human genetic disease

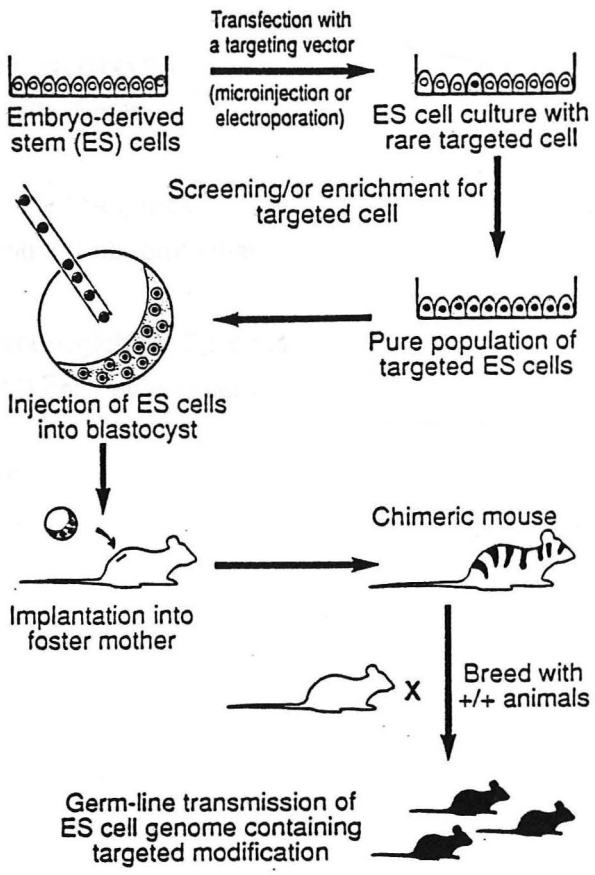
References 5, 6, 8, 9, 69, 70

Figure 8. Gene Targeting by Homologous Recombination: Positive and Negative Selection



A replacement vector for hypothetical gene *X*. The vector contains an insertion of the neomycin resistance gene (*neo*^{r}) in an exon of gene *X* and a linked Herpes simplex virus thymidine kinase (HSV-*tk*) gene. **a**, Homologous recombination between the targeting vector and genomic *X* DNA results in the disruption of one copy of gene *X* and the loss of the HSV-*tk* sequence. Such cells will be resistant to both G418 (resistance conferred by the *neo*^{r} gene) and gancyclovir (which is toxic only to cells expressing the HSV-*tk* gene). **b**, Because non-homologous insertion of exogenous DNA into the genome occurs through the ends of the linearized DNA, the HSV-*tk* gene remains linked to the *neo*^{r} gene. Such cells are resistant to G418 , but sensitive to gancyclovir. Open boxes denote introns or flanking DNA sequences, closed boxes denote exons, and cross-hatch boxes denote the selection markers. From reference 69.

Figure 9. Generation of a Transgenic Mouse Line Containing a Targeted Gene by Transfer of Embryonic Stem Cells



From reference 6

Table XIII. Some Examples of Transgenic Mice with Homozygous Gene Deletions

Deleted Gene: HPRT (X-linked)
Result: Decreased striatal dopamine; clinially normal

Deleted Gene: ß2-microglobulin
Result: Loss of CD8+ T cells

Deleted Gene: Homeobox genes
Result: Developmental abnormalities

Deleted Gene: Immunoglobulin Cμ locus
Result: Failure of B cell development

References 71-75

References

1. Mertz, J. E. and Gurdon, J. B. (1977). Purified DNAs are transcribed after microinjection into *Xenopus* oocytes. **Proc Natl Acad Sci U S A** 74: 1502-6.

2. Palmiter, R. D. and Brinster, R. L. (1986). Germ-line transformation of mice. **Ann Rev Genet** 20: 465-500.

3. Brinster, R. L. and Palmiter, R. D. (1986). Introduction of genes into the germ line of animals. **Harvey Lectures** Series 80: 1-38.

4. Hammer, R. E. (1988). The scientific contributions of Ralph L. Brinster to understanding mammalian embryo development and eukaryotic gene expression. In *Cellular Factors in Development and Differentiation: Embryos, Teratocarcinomas, and Differentiated Tissues,* Harris, S. E., Mansson, P.-E., eds. Alan R. Liss, Inc. pp. 1-30.

5. Jaenisch, R. (1988). Transgenic animals. **Science** 240: 1468-1474.

6. Capecchi, M. R. (1989). The new mouse genetics: altering the genome by gene targeting. **Trends in Genetics** 5: 70-76.

7. Babinet, C., Morello, D., and Renard, J. P. (1989). Transgenic mice. **Genome** 31: 938-949.

8. Schwartzberg, P. L., Goff, S. P., and Robertson, E. J. (1989). Germ-line transmission of a *c-abl* mutation produced by targeted gene disruption in ES cells. **Science** 246: 799-803.

9. Thompson, S., Clarke, A. R., Pow, A. M., Hooper, M. L., and Melton, D. W. (1989). Germ line transmission and expression of a corrected HPRT gene produced by gene targeting in embryonic stem cells. **Cell** 56: 313-321.

10. Mountz, J. D., Zhou, T., and Johnson, L. (1990). Production of transgenic mice and application to immunology and autoimmunity. **Am J Med Sci** 300: 322-9.

11. Brinster, R. L., Chen, H. Y., Trumbauer, M. E., Yagle, M. K., and Palmiter, R. D. (1985). Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. **Proc Natl Acad Sci USA** 82: 4438-4442.

12. Hogan, B., Costantini, F., and Lacy, E. (1986). *Manipulating the Mouse Embryo. A Laboratory Manual.* Cold Spring Harbor Laboratory.

13. Palmiter, R. D., Sandgren, E. P., Avarbock, M. R., Allen, D. D., and Brinster, R. L. (1991). Heterologous introns can enhance expression of transgenes in mice. **Proc**

Natl Acad Sci U S A 88: 478-82.

14. Hanahan, D. (1988). Dissecting multistep tumorigenesis in transgenic mice. **Annu Rev Genet** 22: 479-519.

15. Chisari, F. V., Klopchin, K., Moriyama, T., Pasquinelli, C., Dunsford, H. A., Sell, S., Pinkert, C. A., Brinster, R. L., and Palmiter, R. D. (1989). Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. **Cell** 59: 1145-56.

16. Dunnett, S. B., Sirinathsinghji, D. J., Heavens, R., Rogers, D. C., and Kuehn, M. R. (1989). Monoamine deficiency in a transgenic (Hprt-) mouse model of Lesch-Nyhan syndrome. **Brain Res** 501: 401-6.

17. Pattengale, P. K., Stewart, T. A., Leder, A., Sinn, E., Muller, W., Tepler, I., Schmidt, E., and Leder, P. (1989). Animal models of human disease. Pathology and molecular biology of spontaneous neoplasms occurring in transgenic mice carrying and expressing activated cellular oncogenes. **Am J Pathol** 135: 39-61.

18. Semenza, G. L., Traystman, M. D., Gearhart, J. D., and Antonarakis, S. E. (1989). Polycythemia in transgenic mice expressing the human erythropoietin gene. **Proc Natl Acad Sci U S A** 86: 2301-5.

19. Dent, L. A., Strath, M., Mellor, A. L., and Sanderson, C. J. (1990). Eosinophilia in transgenic mice expressing interleukin 5. **J Exp Med** 172: 1425-31.

20. Greaves, D. R., Fraser, P., Vidal, M. A., Hedges, M. J., Ropers, D., Luzzatto, L., and Grosveld, F. (1990). A transgenic mouse model of sickle cell disorder. **Nature** 343: 183-5.

21. Hammer, R. E., Maika, S. D., Richardson, J. A., Tang, J.-P., and Taurog, J. D. (1990). Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human ß2-m: an animal model of HLA-B27-associated human disorders. **Cell** 63: 1099-1112.

22. Heisterkamp, N., Jenster, G., ten Hoeve, J., Zovich, D., Pattengale, P. K., and Groffen, J. (1990). Acute leukaemia in *bcr/abl* transgenic mice. **Nature** 344: 251-3.

23. Ito, Y., Azrolan, N., OConnell, A., Walsh, A., and Breslow, J. L. (1990). Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. **Science** 249: 790-3.

24. Moriyama, T., Guilhot, S., Klopchin, K., Moss, B., Pinkert, C. A., Palmiter, R. D., Brinster, R. L., Kanagawa, O., and Chisari, F. V. (1990). Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. **Science**

248: 361-4.

25. Muller, W. J., Lee, F. S., Dickson, C., Peters, G., Pattengale, P., and Leder, P. (1990). The *int-2* gene product acts as an epithelial growth factor in transgenic mice. **Embo J** 9: 907-13.

26. Ren, R. B., Costantini, F., Gorgacz, E. J., Lee, J. J., and Racaniello, V. R. (1990). Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. **Cell** 63: 353-62.

27. Ryan, T. M., Townes, T. M., Reilly, M. P., Asakura, T., Palmiter, R. D., Brinster, R. L., and Behringer, R. R. (1990). Human sickle hemoglobin in transgenic mice. **Science** 247: 566-8.

28. Adams, T. E., Alpert, S., and Hanahan, D. (1987). Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. **Nature** 325: 223-228.

29. Allison, J., Campbell, I. L., Morahan, G., Mandel, T. E., Harrison, L. C., and Miller, J. F. A. P. (1988). Diabetes in transgenic mice resulting from over-expression of class I histocompatibility molecules in pancreatic beta cells. **Nature** 333: 529-533.

30. Lo, D., Burkly, L. C., Widera, G., Cowing, C., Flavell, R. A., Palmiter, R. D., and Brinster, R. L. (1988). Diabetes and tolerance in transgenic mice expressing class II MHC molecules in pancreatic beta cells. **Cell** 53: 159-168.

31. Sarvetnick, N., Liggitt, D., Pitts, S. L., Hansen, S. E., and Stewart, T. A. (1988). Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. **Cell** 52: 773-782.

32. Bohme, J., Haskins, K., Stecha, P., van Ewijk, W., LeMeur, M., Gerlinger, P., Benoist, C., and Mathis, D. (1989). Transgenic mice with I-A on islet cells are normoglycemic but immunologically intolerant. **Science** 244: 1179-83.

33. Epstein, P. N., Overbeek, P. A., and Means, A. R. (1989). Calmodulin-induced early-onset diabetes in transgenic mice. **Cell** 58: 1067-73.

34. Morahan, G., Allison, J., and Miller, J. F. (1989). Tolerance of class I histocompatibility antigens expressed extrathymically. **Nature** 339: 622-4.

35. Lipes, M. A. and Eisenbarth, G. S. (1990). Transgenic mouse models of type I diabetes. **Diabetes** 39: 879-84.

36. Roman, L. M., Simons, L. F., Hammer, R. E., Sambrook, J. F., and Gething, M. J.

(1990). The expression of influenza virus hemagglutinin in the pancreatic beta cells of transgenic mice results in autoimmune diabetes. **Cell** 61: 383-96.

37. Sarvetnick, N., Shizuru, J., Liggitt, D., Martin, L., McIntyre, B., Gregory, A., Parslow, T., and Stewart, T. (1990). Loss of pancreatic islet tolerance induced by beta-cell expression of interferon-gamma. **Nature** 346: 844-7.

38. Ohashi, P. S., Oehen, S., Buerkl, K., Pircher, H., Ohashi, C. T., Odermatt, B., Malissen, B., Zinkernagel, R. M., and Hengartner, H. (1991). Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. **Cell** 65: 305-317.

39. Oldstone, M. B. A., Nerenberg, M., Southern, P., Price, J., and Lewicki, H. (1991). Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. **Cell** 65: 319-331.

40. Hammer, R. E., Palmiter, R. D., and Brinster, R. L. (1984). Partial correction of murine hereditary disorder by germ-line incorporation of a new gene. **Nature** 311: 65-67.

41. Readhead, C., Popko, B., Takahashi, N., Shine, H. D., Saavedra, R. A., Sidman, R. L., and Hood, L. (1987). Expression of a myelin basic protein gene in transgenic *shiverer* mice: correction of the dysmyelinating phenotype. **Cell** 48: 703-12.

42. Jones, S. N., Grompe, M., Munir, M. I., Veres, G., Craigen, W. J., and Caskey, C. T. (1990). Ectopic correction of ornithine transcarbamylase deficiency in *sparse fur* mice. **J Biol Chem** 265: 14684-90.

43. Yokoyama, T., Silversides, D. W., Waymire, K. G., Kwon, B. S., Takeuchi, T., and Overbeek, P. A. (1990). Conserved cysteine to serine mutation in tyrosinase is responsible for the classical albino mutation in laboratory mice. **Nucleic Acids Res** 18: 7293-8.

44. Bruggemann, M., Caskey, H. M., Teale, C., Waldmann, H., Williams, G. T., Surani, M. A., and Neuberger, M. S. (1989). A repertoire of monoclonal antibodies with human heavy chains from transgenic mice. **Proc Natl Acad Sci U S A** 86: 6709-13.

45. Archibald, A. L., McClenaghan, M., Hornsey, V., Simons, J. P., and Clark, A. J. (1990). High-level expression of biologically active human alpha 1-antitrypsin in the milk of transgenic mice. **Proc Natl Acad Sci U S A** 87: 5178-82.

46. Arnheiter, H., Skuntz, S., Noteborn, M., Chang, S., and Meier, E. (1990). Transgenic mice with intracellular immunity to influenza virus. **Cell** 62: 51-61.

47. Wilmut, I., Archibald, A. L., Harris, S., McClenaghan, M., Simons, J. P., Whitelaw, C. B., and Clark, A. J. (1990). Modification of milk composition. **J Reprod Fertil Suppl** 41: 135-46.

48. Mickisch, G. H., Merlino, G. T., Galski, H., Gottesman, M. M., and Pastan, I. (1991). Transgenic mice that express the human multidrug-resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. **Proc Natl Acad Sci U S A** 88: 547-51.

49. Adams, E., Basten, A., and Goodnow, C. C. (1990). Intrinsic B-cell hyporesponsiveness accounts for self-tolerance in lysozyme/anti-lysozyme double-transgenic mice. **Proc Natl Acad Sci U S A** 87: 5687-91.

50. Adams, T. E. (1990). Tolerance to self-antigens in transgenic mice. **Mol Biol Med** 7: 341-57.

51. Arnold, B., Goodnow, C., Hengartner, H., and Hämmerling, G. (1990). The coming of transgenic mice: tolerance and immune reactivity. **Immunol Today** 11: 69-72.

52. Goodnow, C. C., Adelstein, S., and Basten, A. (1990). The need for central and peripheral tolerance in the B cell repertoire. **Science** 248: 1373-9.

53. Hanahan, D. (1990). Transgenic mouse models of self-tolerance and autoreactivity by the immune system. **Annu Rev Cell Biol** 6: 493-537.

54. Miller, J. F., Morahan, G., and Allison, J. (1989). Immunological tolerance: new approaches using transgenic mice. **Immunol Today** 10: 53-7.

55. Pircher, H., Burki, K., Lang, R., Hengartner, H., and Zinkernagel, R. M. (1989). Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. **Nature** 342: 559-61.

56. von Boehmer, H., Teh, H. S., and Kisielow, P. (1989). The thymus selects the useful, neglects the useless and destroys the harmful. **Immunol Today** 10: 57-61.

57. von Boehmer, H. (1990). Self recognition by the immune system. **Eur J Biochem** 194: 693-698.

58. Chada, K., Magram, J., and Costantini, F. (1986). An embryonic pattern of expression of a human fetal globin gene in transgenic mice. **Nature** 319: 685-9.

59. Costantini, F., Chada, K., and Magram, J. (1986). Correction of murine beta-

thalassemia by gene transfer into the germ line. **Science** 233: 1192-4.

60. Grosveld, F., van Assendelft, G. B., Greaves, D. R., and Kollias, B. (1987). Position-independent, high-level expression of the human beta-globin gene in transgenic mice. **Cell** 51: 975-985.

61. Behringer, R. R., Ryan, T. M., Reilly, M. P., Asakura, T., Palmiter, R. D., Brinster, R. L., and Townes, T. M. (1989). Synthesis of functional human hemoglobin in transgenic mice. **Science** 245: 971-3.

62. Behringer, R. R., Ryan, T. M., Palmiter, R. D., Brinster, R. L., and Townes, T. M. (1990). Human gamma- to beta-globin gene switching in transgenic mice. **Genes Dev** 4: 380-9.

63. Enver, T., Raich, N., Ebens, A., Papayannopoulou, T., Constantini, F., and Stamatoyannopoulos, G. (1990). Developmental regulation of human fetal-to-adult globin gene switching in transgenic mice. **Nature** 344: 309-313.

64. Higgs, D. R., Wood, W. G., Jarman, A. P., Sharpe, J., Lida, J., Pretorius, I. M., and Ayyub, H. (1990). A major positive regulatory region located far upstream of the human alpha-globin gene locus. **Genes Dev** 4: 1588-601.

65. Orkin, S. H. (1990). Globin gene regulation and switching: circa 1990. **Cell** 63: 665-72.

66. Papayannopoulou, T., Brice, M., Enver, T., and Stamatoyannopoulos, G. (1990). Globin gene switching: insights from studies in somatic heterospecific hybrids and in transgenic mice. **Prog Clin Biol Res** 352: 323-8.

67. Townes, T. M. and Behringer, R. R. (1990). Human globin locus activation region (LAR): role in temporal control. **Trends Genet** 6: 219-23.

68. Stamatoyannopoulos, G. (1991). Human hemoglobin switching. **Science** 252: 383.

69. Mansour, S. L., Thomas, K. R., and Capecchi, M. R. (1988). Disruption of the proto-oncogene *int-2* in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. **Nature** 336: 348-352.

70. Baribault, H. and Kemler, R. (1989). Embryonic stem cell culture and gene targeting in transgenic mice. **Mol Biol Med** 6: 481-92.

71. Kuehn, M. R., Bradley, A., Robertson, E. J., and Evans, M. J. (1987). A potential animal model for Lesch-Nyhan syndrome through introduction of HPRT mutations into

mice. **Nature** 326: 295-8.

72. Zijlstra, M., Li, E., Sajjadi, F., Subramani, S., and Jaenisch, R. (1989). Germ-line transmission of a disrupted beta 2-microglobulin gene produced by homologous recombination in embryonic stem cells. **Nature** 342: 435-8.

73. Koller, B. H., Marrack, P., Kappler, J. W., and Smithies, O. (1990). Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8+ T cells. **Science** 248: 1227-30.

74. Chisaki, O. and Capecchi, M. R. (1991). Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *hox-1.5*. **Nature** 350: 473-7.

75. Kitamura, D., Roes, J., Kuehn, R., and Rajewsky, K. (1991). A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. **Nature** 350: 423-5.