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Cellular DNA Rearrangements and Early Developmental Arrest Caused by DNA Insertion in Transgenic Mouse Embryos

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Insertional mutagenesis was investigated in a transgenic mouse strain (HUGH/4) derived from a fertilized egg injected with plasmid DNA containing the human growth hormone gene. Lethality occurred in homozygous embryos and was traced to the egg cylinder stage on days 4 to 5 of gestation, shortly after implantation. The mutation is on chromosome 12 and is distinct in location and integration pattern from another mutation also leading to lethality of homozygotes in the egg cylinder stage. Based on this and other evidence, relatively many genes may be recruited to activity near the time of implantation and may therefore present a large target of vulnerability to mutagenesis. The single insert in HUGH/4, consisting of approximately three tandem copies of plasmid sequences, is flanked by mouse cellular sequences that have undergone rearrangements, including a probable deletion. The data suggest the hypothesis that DNA rearrangements, which appear to be commonplace in transgenic mice, may arise because the initial insertional complex is unstable; stepwise changes may then be generated until a more stable conformation is achieved.

The observation that two of a group of six transgenic mouse strains failed to include homozygotes among the newborns (31) led us to search for the developmental and molecular basis of the lethalities. Although each strain was heterozygous for phGH plasmid DNA containing the human growth hormone gene and pBR322 sequences (8), the two insertional mutations were independent. In the first strain to be studied (HUGH/3), homozygous embryos died shortly after implantation in the egg cylinder stage on days 4 to 5 of gestation, because of extensive rearrangements and a probable deletion in the mouse flanking DNA regions (5, 6). In studying the other strain (HUGH/4), as reported here, we were surprised to find the homozygotes dying in the same period and rearrangements again occurring in neighboring cellular DNA, albeit in a different pattern and on a different chromosome. Taken together, along with other evidence from transgenic mice, these cases suggest that rearrangements caused by DNA integration are fairly common. Implications for the mechanics of insertion and for gene expression in early stages of mammalian development are proposed.

MATERIALS AND METHODS

Molecular cloning and restriction analysis. The procedures described in detail in the HUGH/3 study (6) were followed here. High-molecular-weight DNA was prepared (11, 37) from HUGH/4 progeny identified as heterozygotes (31) and from controls of the C3H, C57BL/6, and BALB/c mouse strains. Partial libraries of DNAs (17) from HUGH/4 were used to isolate *Eco*RI restriction fragments containing mouse chromosomal sequences flanking the phGH insert. Clones prepared in λgtWES were screened (8, 31) and subcloned in pUC9. Restriction analysis was done by standard techniques (27, 33).

Chromosomal location of inserted DNA. Filters containing transfers of *Eco*RI-digested and electrophoresed DNA were prepared from mouse-Chinese hamster hybrid cell lines characterized by their content of specific mouse chromosomes (7). The filters were probed for hybridization with cloned fragments of the regions flanking the phGH insert in HUGH/4 mice.

RESULTS

Stage of lethality of homozygous embryos. A total of 118 embryos from *HGH*/+ × *HGH*/+ matings were examined on days 3 through 12 of gestation (counting the vaginal plug date as day 0) and compared with controls. In the experimental matings, the numbers of preimplantation embryos on day 3 were normal (16 embryos in two litters), and all were blastocysts microscopically indistinguishable from normal controls. After implantation, the numbers of uterine decidual sites, indicative of implantation, were normal (average 6.5 to 10 per female, except for 5 in one female), but approximately one-fourth of the sites—accounting for the expected frequency of mutant homozygotes—were devoid of embryos or contained dead embryos undergoing resorption. This included 25 unequivocal defectives of 94 total sites on days 6 to 12 (Table 1), verified at the youngest stages by serial sectioning at 8 μm in utero and staining with hematoxylin and eosin. (Of an additional 24 sites, including 16 on day 5

TABLE 1. Frequency of lethal HUGH/4 embryos from matings of *HGH*/+ × *HGH*/+

Day of gestation	No. defective[a]/ total	% Defective
3	0/16	0
6	2/10	20
7	5/13	38
8	3/14	21
9	3/9	33
10	6/10	60
11	2/5	40
12	4/17	24

[a] Abnormal or dead embryo or decidual site lacking an embryo.

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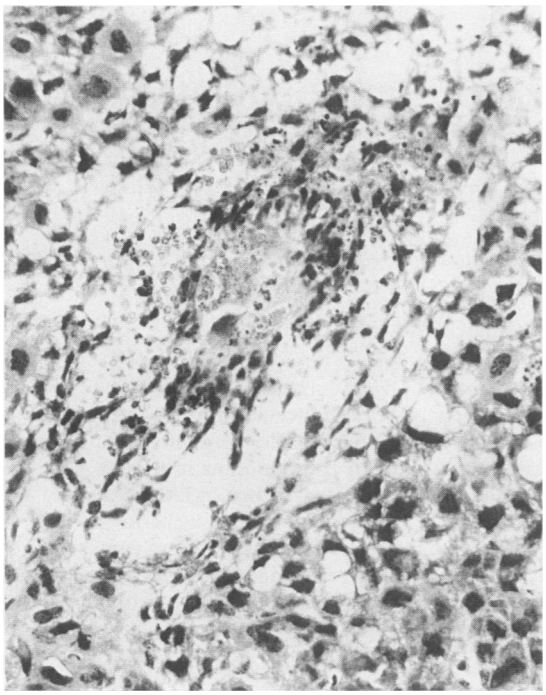

FIG. 1. Histological section of a lethal HUGH/4 embryo from a mating of *HGH*/+ × *HGH*/+ on day 6 of gestation. The embryo had died at least a day earlier, in the egg cylinder stage after implantation. Hematoxylin and eosin stain, 8-μm section.

and 8 on day 6, some appeared to contain only traces of embryos but were not sectioned.) From the histological appearance of serial sections on day 6 (Fig. 1), development of the mutants was blocked in the egg cylinder stage, and death must have occurred shortly after implantation, no later than day 5.

DNA clones flanking the insert. Approximately three tandem copies of phGH sequences are present in HUGH/4 and integration has occurred within the human gene, resulting in two intact copies of phGH and three copies of pBR322 (31). Genomic DNA from this strain digested with *Eco*RI hybridizes to the phGH probe in four fragments: 4.3 kilobases (kb) (pBR322) and 3.4, 2.8, and 2.6 kb (hGH). The largest and smallest fragments include multiple copies of the plasmid sequences. The remaining two fragments are expected to contain the junctional regions with both mouse and human sequences (Fig. 2 and 3). Of the two flanking-sequence fragments, the 3.4-kb fragment was cloned.

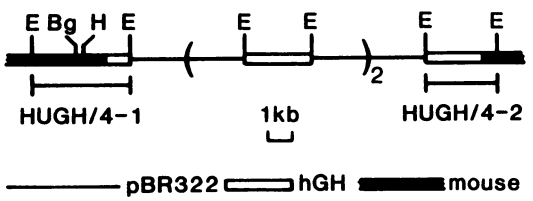

FIG. 2. The phGH integration map of HUGH/4, based on Southern blot analyses of single and double digests of DNA hybridized with labeled hGH or pBR322 sequences. There are two intact tandem copies of the phGH plasmid (shown in parentheses). Two *Eco*RI fragments (HUGH/4-1 and HUGH/4-2) flanking the phGH sequences are indicated. The HUGH/4-2 region was not cloned, so that the size of the mouse region within the 2.8-kb *Eco*RI fragment is unknown. Abbreviations: E, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII.

HUGH/4 genomic DNA was digested with *Eco*RI, and the fraction containing the candidate flanking region was ligated to λgtWES *Eco*RI arms. The partial library of approximately 5×10^5 clones was screened with a nick-translated phGH probe, and a positive clone, designated λHUGH/4-1 (Fig. 3), was isolated. This was subcloned into the *Eco*RI site of pUC9, yielding pHUGH/4-1. Hybridization confirmed the presence of mouse and human sequences adjacent to an *Eco*RI site. Repetitive sequences (unidentified) were found when nick-translated wild-type genomic DNA was hybridized to restriction fragments immobilized on nitrocellulose (data not shown). Under the conditions used, only sequences present in over 100 copies per genome could be detected (28). Their location is shown in Fig. 3. The 2.2-kb *Hin*dIII-*Eco*RI fragment from pHUGH/4-1 lacks repetitive sequences and was subcloned into pUC9 to yield pHUGH/4-1Δ.

Host sequences rearranged by plasmid DNA insertion. Southern blots were prepared from *Eco*RI-restricted genomic DNA of the C3H and C57BL/6 inbred strains (the parental strains of the HUGH/4 founder animal) and of the BALB/c strain. The blots were tested for hybridization with the pHUGH/4-1Δ clone. Of the two *Eco*RI flanking fragments (3.4 and 2.8 kb), approximately 3 to 5 kb is mouse DNA and the rest is phGH sequences. If HUGH/4 had been generated by a simple single-step insertion into wild-type DNA, as implied by the integration pattern (Fig. 2), then the pHUGH/4-1Δ probe should hybridize to a single 3- to 5-kb *Eco*RI fragment in C3H or C57BL/6 DNA. However, pHUGH/4-1Δ hybridizes to two *Eco*RI fragments (8.6 and 1.6 kb) in wild-type DNA (Fig. 4), although the probe has no internal *Eco*RI site. Similarly, this probe hybridizes to three *Hin*dIII fragments although there is no internal *Hin*dIII site in the probe (Fig. 4). Further discrepancies are apparent for *Pst*I, *Pvu*II, and *Kpn*I; only *Bgl*II gave results consistent with the restriction map of λHUGH/4-1 (Fig. 3). From restriction patterns of the control strains, restriction-site

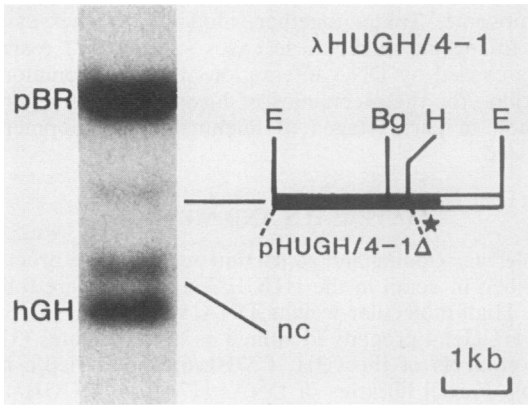

FIG. 3. Origin of cloned mouse flanking sequences. The Southern blot shows the *Eco*RI pattern of HUGH/4 DNA hybridized with nick-translated phGH. Of the two *Eco*RI flanking-sequence fragments represented by 3.4- and 2.8-kb bands, the 2.8-kb fragment was not cloned (nc). The restriction map for the 3.4-kb fragment (λHUGH/4-1) is shown, with a star in the position of repetitive sequences; sites for *Pst*I, *Pvu*II, and *Kpn*I are absent. pHUGH/4-1Δ is a subclone from which repetitive sequences were removed. Restriction sites within hGH or pBR322 are omitted. Abbreviations: E, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII.

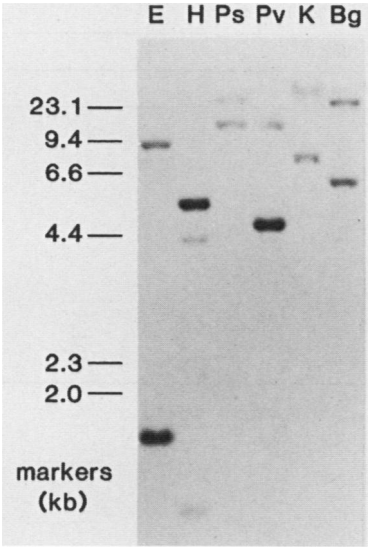

FIG. 4. Southern blot analyses of wild-type genomic DNA digests hybridized with the pHUGH/4-1Δ flanking-sequence probe. Note that for each of the restriction enzymes shown, except for *Bgl*II, the number of hybridizable fragments is not as expected from the restriction map of the λHUGH/4-1 clone. Abbreviations: E, *Eco*RI; H, *Hin*dIII; Ps, *Pst*I; Pv, *Pvu*II; K, *Kpn*I; Bg, *Bgl*II.

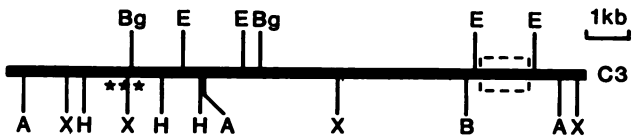

FIG. 5. Restriction map of wild-type target sequences. A wild-type genomic clone (C3) was isolated by using pHUGH/4-1Δ as a probe. The clone was found to have a small region (-----) homologous to the probe and consistent with the presence of a 1.6-kb *Eco*RI fragment in the Southern blot of wild-type DNA (Fig. 4). The region spanned by the C3 clone accounts for some of the hybridizable bands in the Southern blot analysis of wild-type DNA (see the text). E, *Eco*RI; H, *Hin*dIII; A, *Ava*I; X, *Xba*I; B, *Bam*HI; Bg, *Bgl*II; ★★★, repetitive sequences.

polymorphisms are not likely to account for the disparities (data not shown). (The phGH probe did not hybridize to the mouse growth hormone gene under the conditions used here.)

The results therefore indicate that a series of events involving deletions or rearrangements (or both) of host DNA sequences, rather than a simple insertion, occurred during integration of the plasmid DNA. Although examination of metaphase spreads from HUGH/4 heterozygotes did not disclose any karyotypic anomalies (data not shown), translocation of some sequences from other regions cannot be ruled out.

Wild-type and HUGH/4 flanking sequences. With pHUGH/4-1Δ as a probe, four similar clones were independently isolated from a wild-type genomic library. Restriction mapping followed by Southern blotting analysis of one of the clones, C3, revealed that the DNA region that hybridized to pHUGH/4-1Δ was within the 1.6-kb *Eco*RI fragment shown in Fig. 5 and present in the Southern blot shown in Fig. 4. The restriction map of C3 is also consistent with the occurrence of some of the hybridizable bands in the total Southern blot analysis of wild-type DNA with *Ava*I and *Xba*I digests (data not shown).

However, Southern blot analysis of wild-type DNA also reveals extra bands which clearly do not originate from the C3 region. Furthermore, the repetitive sequences found in pHUGH/4-1 do not neighbor the 1.6-kb *Eco*RI fragment of C3 DNA. These facts support the conclusion that rearrangements have occurred in the cellular sequences flanking the phGH integration.

Chromosomal location of the insert. *Eco*RI-digested DNA was prepared from a number of mouse-Chinese hamster hybrid cell lines characterized by limited numbers of mouse chromosomes (7). After DNA electrophoresis and blotting, the filter was hybridized with clone pHUGH/4-1Δ DNA. The pattern of hybridization indicates that the plasmid sequences were integrated on chromosome 12 (Table 2). This is particularly evident from the hybridization to DNA fragments from the cell line MAE28, which contains only mouse chromosomes 12 and X, and from the lack of hybridization to DNA fragments from MAE32, which contains only mouse chromosomes 16 and X. A DNA probe from the plasmid pV_HJ558 (a gift from Peter Brodeur and Roy Riblet) served as a positive control because it includes immunoglobulin heavy-chain variable-region sequences known to be present on chromosome 12. Under the stringent washing conditions employed, no hybridization was observed with Chinese hamster DNA.

DISCUSSION

Frequency of early developmental mutations among transgenic mice. The present case of insertional mutagenesis clearly differs from the HUGH/3 case (5, 31) in DNA changes and in chromosomal location (identified as on number 12 in HUGH/4, unknown but not on number 12 in HUGH/3). The double heterozygotes between the two strains are normal (31). Yet each mutation is a recessive lethal gene leading to developmental arrest on days 4 to 5 in the egg cylinder stage soon after implantation. Moreover, both are characterized by host DNA rearrangements, including deletions, in regions bordering the insertions.

Other insertional mutations, including nonlethal ones, have recently been described in experimental transgenic mice (10, 18, 22, 23, 26, 35). We estimated that such mutations constitute a strikingly high frequency (approximately 15 to 20%) of cases adequately screened (5). It is noteworthy that another of these causes early postimplantation lethality (18). One of two probable cases of mutation due to spontaneous retroviral insertion (4, 13), involving the lethal yellow (A^y) gene, also results in recessive lethality at about the time of implantation (4).

From the foregoing evidence, we propose that the embryo shortly after implantation is especially vulnerable to adverse genetic changes because of transcriptional activation of many genetic loci as a normal prerequisite for further development. Of various genetic regions into which DNA may become inserted in the zygote stage, those loci destined to be expressed near the time of implantation would constitute a relatively large target size. When a much larger sample of insertional mutations is collected in transgenic mice, it would thus be expected to include a disproportionately large number causing early-postimplantation defects. On the same principle, discrete time clusters of deleterious mutations may also be discovered at certain later (currently unpredictable) stages, representing windows of development characterized by de novo transcription of many loci.

The known mutations among transgenic mice provide indirect evidence that when donor DNA sequences were

TABLE 2. Chromosomal localization of the phGH integration site[a]

Mouse chromosome or probe	Presence of chromosome in:										
			Hybrid cell lines								
	Mouse control (C57BL/6 liver)	Chinese hamster control (E36)	MACH4A63	MACH4B31Az3	MACH2A2B1	MACH2A2C2	MACH2A2H3	MAE28	MAE32	R44-1	ECm4e
Mouse chromosome											
1	+	−	−	−	+	+	+	−	−	−	−
2	+	−	+	+	+	+	−	−	−	−	−
3	+	−	−	−	+	+	+	−	−	−	−
4	+	−	−	−	+	±[b]	±	−	−	−	−
5	+	−	−	−	−	−	−	−	−	−	−
6	+	−	−	−	+	−	+	−	−	−	−
7	+	−	+	+	+	+	+	−	−	−	−
8	+	−	−	+	+	+	+	−	−	−	−
9	+	−	−	−	+	+	+	−	−	−	−
10	+	−	−	−	+	+	+	−	−	−	−
11	+	−	−	−	−	−	−	−	−	−	−
12	+	−	±	±	+	+	+	+	−	−	−
13	+	−	+	−	−	+	+	−	−	−	−
14	+	−	−	±	+	−	+	−	−	−	+
15	+	−	+	±	+	+	+	−	−	−	+
16	+	−	+	+	+	+	+	−	+	−	−
17	+	−	+	+	+	+	+	−	−	+	−
18	+	−	+	−	−	−	+	−	−	−	−
19	+	−	+	+	−	+	+	−	−	−	−
X	+	−	−	−	+	+	+	+	+	−	−
Probe											
pHUGH/4-1Δ	+	−	+	+	+	+	+	+	−	−	−
pV_HJ558	+	−	+	+	+	+	+	+	−	−	−

[a] Distribution of mouse chromosomes in the designated mouse-Chinese hamster hybrid cell lines was previously determined karyotypically.
[b] Less than 20% of the cells contain the mouse chromosome(s).

integrated in the zygote they did not preferentially enter regions actively transcribing at that time. Nevertheless, integration does not necessarily occur at random sites. We have suggested that recombinatorial "hotspots" may exist for insertion of exogenous DNA (6), irrespective of whether the regions are then transcriptionally active. Such hotspots may be related to specific chromosomal fragile sites (38) or may be comparable to regions of naturally occurring rearrangements such as those in the major histocompatibility complex (29) or in immunoglobulin gene regions.

The possibility that many loci may begin to be expressed near the time of implantation is consistent with the following points: stable maternal message is present in the mouse egg but is appreciable only during preimplantation development (3, 19, 25), paternal alleles of certain enzymes are not expressed before the late blastocyst stage (2), the paternal genome is required for the development of the extraembryonic tissues (1), and founder cells for many specific cell types appear to be determined, or set aside, in the early postimplantation stages (20).

Frequency of host DNA rearrangements induced by insertion of DNA sequences. It is apparent from many Southern blots illustrated in the literature on transgenic mice that host cellular DNA rearrangements caused by plasmid DNA integration must occur frequently, albeit often without generating a mutant phenotype. The evidence is the presence of excess numbers of fragments that hybridize to the plasmid probe. An example in a mutation affecting limb development involved a deletion of approximately 1 kb of flanking mouse sequences (35). Changes in host as well as inserted DNAs have also been described in cultured somatic cells (12, 14, 16, 21, 30).

The present results reinforce the view stated previously (6) that integration of DNA into the mammalian genome may occur as a complex series of events. The first product of DNA integration may often be a highly unstable structure. Rearrangements, in the insert as well as in the host target sequences, then follow as a means of attaining a more stable conformation. In the integration of plasmid (but not retroviral) DNA into the mouse egg, the plasmid sequences are usually found in a tandem array. Although it is not known whether this occurs by homologous recombination before integration (9) or by integration followed by some form of gene amplification (24, 32), there must be a series of discrete steps in the integration process, apart from actual ligation of donor to host DNA.

As others have pointed out, topological variants of DNA structure frequently characterize DNA recombination (15, 34, 36). Among factors that might influence DNA conformation and stability is the occurrence of new tracts of base sequences resulting from tandemization, from association of exogenous and endogenous DNAs, or from local amplifica-

tion. Thus, whereas the mammalian genome appears to be surprisingly labile in admitting new DNA sequences, the process is likely often to be mutagenic.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Barton, S. C., M. A. H. Surani, and M. L. Norris.** 1984. Role of paternal and maternal genomes in mouse development. Nature (London) **311**:374–376.
2. **Chapman, V. M., W. K. Whitten, and F. H. Ruddle.** 1971. Expression of paternal glucose phosphate isomerase-1 (Gpi-1) in preimplantation stages of mouse embryos. Dev. Biol. **26**:153–158.
3. **Clegg, K. B., and L. Pikó.** 1983. Poly(A) length, cytoplasmic adenylation and synthesis of poly(A)$^+$ RNA in early mouse embryos. Dev. Biol. **95**:331–341.
4. **Copeland, N. G., N. A. Jenkins, and B. K. Lee.** 1983. Association of the lethal yellow (A^y) coat color mutation with an ecotropic murine leukemia virus genome. Proc. Natl. Acad. Sci. USA **80**:247–249.
5. **Covarrubias, L., Y. Nishida, and B. Mintz.** 1985. Early developmental mutations due to DNA rearrangements in transgenic mouse embryos. Cold Spring Harbor Symp. Quant. Biol. **50**:447–452.
6. **Covarrubias, L., Y. Nishida, and B. Mintz.** 1986. Early postimplantation embryo lethality due to DNA rearrangements in a transgenic mouse strain. Proc. Natl. Acad. Sci. USA **83**:6020–6024.
7. **D'Eustachio, P., A. L. M. Bothwell, T. K. Takaro, D. Baltimore, and F. H. Ruddle.** 1981. Chromosomal location of structural genes encoding murine immunoglobulin λ light chains: genetics of murine λ light chains. J. Exp. Med. **153**:793–800.
8. **Fiddes, J. C., P. H. Seeburg, F. M. De Noto, R. A. Hallewell, J. D. Baxter, and H. M. Goodman.** 1979. Structure of genes for human growth hormone and chorionic somatomammotropin. Proc. Natl. Acad. Sci. USA **76**:4294–4298.
9. **Folger, K. R., E. A. Wong, G. Wahl, and M. R. Capecchi.** 1982. Patterns of integration of DNA microinjected into cultured mammalian cells: evidence for homologous recombination between injected plasmid DNA molecules. Mol. Cell. Biol. **2**:1372–1387.
10. **Gordon, J. W.** 1986. A foreign dihydrofolate reductase gene in transgenic mice acts as a dominant mutation. Mol. Cell. Biol. **6**:2158–2167.
11. **Grosschedl, R., D. Weaver, D. Baltimore, and F. Costantini.** 1984. Introduction of a μ immunoglobulin gene into the mouse germ line: specific expression in lymphoid cells and synthesis of functional antibody. Cell **38**:647–658.
12. **Hawley, R. G., M. J. Shulman, H. Murialdo, D. M. Gibson, and N. Hozumi.** 1982. Mutant immunoglobulin genes have repetitive DNA elements inserted into their intervening sequences. Proc. Natl. Acad. Sci. USA **79**:7425–7429.
13. **Jenkins, N. A., N. G. Copeland, B. A. Taylor, and B. K. Lee.** 1981. Dilute (*d*) coat colour mutation of DBA/2J mice is associated with the site of integration of an ectotropic MuLV genome. Nature (London) **293**:370–374.
14. **King, W., M. D. Patel, L. I. Lobel, S. P. Goff, and M. C. Nguyen-Huu.** 1985. Insertion mutagenesis of embryonal carcinoma cells by retroviruses. Science **228**:554–558.
15. **Koo, H.-S., H.-M. Wu, and D. M. Crothers.** 1986. DNA bending at adenine · thymine tracts. Nature (London) **320**:501–506.
16. **Lebkowski, J. S., R. B. DuBridge, E. A. Antell, K. S. Greisen, and M. P. Calos.** 1984. Transfected DNA is mutated in monkey, mouse, and human cells. Mol. Cell. Biol. **4**:1951–1960.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Mark, W. H., K. Signorelli, and E. Lacy.** 1985. An insertional mutation in a transgenic mouse line results in developmental arrest at day 5 of gestation. Cold Spring Harbor Symp. Quant. Biol. **50**:453–463.
19. **Mintz, B.** 1964. Synthetic processes and early development in the mammalian egg. J. Exp. Zool. **157**:85–100.
20. **Mintz, B.** 1971. Clonal basis of mammalian differentiation. Symp. Soc. Exp. Biol. **25**:345–370.
21. **Mounts, P., and T. J. Kelly, Jr.** 1984. Rearrangements of host and viral DNA in mouse cells transformed by simian virus 40. J. Mol. Biol. **177**:431–460.
22. **Overbeek, P. A., S.-P. Lai, K. R. Van Quill, and H. Westphal.** 1985. Tissue-specific expression in transgenic mice of a fused gene containing RSV terminal sequences. Science **231**:1574–1577.
23. **Palmiter, R. D., T. M. Wilkie, H. Y. Chen, and R. L. Brinster.** 1984. Transmission distortion and mosaicism in an unusual transgenic mouse pedigree. Cell **36**:869–877.
24. **Roberts, J. M., L. B. Buck, and R. Axel.** 1983. A structure for amplified DNA. Cell **33**:53–63.
25. **Sawicki, J. A., T. Magnuson, and C. J. Epstein.** 1981. Evidence for expression of the paternal genome in the two-cell mouse embryo. Nature (London) **294**:450–451.
26. **Schnieke, A., K. Harbers, and R. Jaenisch.** 1983. Embryonic lethal mutation in mice induced by retrovirus insertion into the α1(I) collagen gene. Nature (London) **304**:315–320.
27. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
28. **Steinmetz, M., J. Höchtl, H. Schnell, W. Gebhard, and H. G. Zachau.** 1980. Cloning of V region fragments from mouse liver DNA and localization of repetitive DNA sequences in the vicinity of immunoglobulin gene segments. Nucleic Acids Res. **8**:1721–1729.
29. **Steinmetz, M., D. Stephan, and K. F. Lindahl.** 1986. Gene organization and recombinational hotspots in the murine major histocompatibility complex. Cell **44**:895–904.
30. **Varmus, H. E., N. Quintrell, and S. Ortiz.** 1981. Retroviruses as mutagens: insertion and excision of a nontransforming provirus alter expression of a resident transforming provirus. Cell **25**:23–26.
31. **Wagner, E. F., L. Covarrubias, T. A. Stewart, and B. Mintz.** 1983. Prenatal lethalities in mice homozygous for human growth hormone gene sequences integrated in the germ line. Cell **35**:647–655.
32. **Wahl, G. M., R. B. de Saint Vincent, and M. L. DeRose.** 1984. Effect of chromosomal position on amplification of transfected genes in animal cells. Nature (London) **307**:516–520.
33. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.
34. **Wasserman, S. A., and N. R. Cozzarelli.** 1986. Biochemical topology: applications to DNA recombination and replication. Science **232**:951–960.
35. **Woychik, R. P., T. A. Stewart, L. G. Davis, P. D'Eustachio, and P. Leder.** 1985. An inherited limb deformity created by insertional mutagenesis in a transgenic mouse. Nature (London) **318**:36–40.
36. **Wu, H. M., and D. M. Crothers.** 1984. The locus of sequence-directed and protein-induced DNA bending. Nature (London) **308**:509–513.
37. **Yaoita, Y., and T. Honjo.** 1980. Deletion of immunoglobulin heavy chain genes accompanies the class switch rearrangement. Biomed. Res. **1**:164–175.
38. **Yunis, J. J., and A. L. Soreng.** 1984. Constitutive fragile sites and cancer. Science **226**:1199–1204.