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GENETIC MOSAICISM IN THE HOUSE MOUSE

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INTRODUCTION

The discovery of phenotypically abnormal individuals is interesting to the casual observer and the trained geneticist alike. The isolation and breeding studies of such mutants lie at the very basis of genetic analysis. Even more interesting is the rare occurrence of individuals with an apparent mixture of phenotypes. Such variegated phenotypes may be of genetic origin as the result of mutations that are expressed per se as a variegated phenotype, functional mosaicism due to heterozygosity for an X-linked trait in mammals, or genetic mosaicism. Genetic mosaics are multicellular organisms comprised of cells of different genotypes. Mosaics may arise by the development of individuals from

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more than a single fertilization event (designated chimeras) or due to the fixation of a mutation following fertilization that is inherited in a fraction of the cells of an organism. Presumed genetic mosaics usually have been identified as individuals expressing a variegated phenotype. Confirmation of mosaicism has required breeding studies and, therefore, involvement of the germ cells. However, the recent advances in molecular biological techniques now allow direct genotyping of even a small number of cells.

Genetic mosaicism has been widely documented (119). The mouse is an ideal laboratory organism with which to study genetic mosaics since a large number of coat-color loci have been identified and genotypically characterized (77, 117) that allow an immediate identification of variegated phenotype. In this review we consider the occurrence of genetic mosaics in the mouse due to mutation and identify parallel situations in humans.

DEVELOPMENTAL CONSIDERATIONS

For our purposes ontogenetic development may be considered a unidirectional branching process consisting of cellular differentiation, proliferation, and migration. Given the occurrence of a mutation during the development of a multicellular organism, the variety and number of organs, tissues, and cells that eventually carry the mutation will depend upon the developmental stage and cell lineage in which the original mutation was fixed.

During the early stages of mouse development, the inner cell mass appears as an aggregation of cells at the 32-cell stage blastocyst. The number of cells from the inner cell mass that contribute to the development of the embryo has been estimated to be as small as three (42, 80, 85). Coat-pigmentation cells develop from 34 primordial melanoblasts (84) derived from the neural crest and are of ectodermal origin. The pigment-producing cells (melanocytes) of the hair follicles covering the body result from the clonal expansion and medio-lateral migration of the primordial melanoblasts, which takes place between days 8 and 12 of gestation (99). The germ cells develop from a considerably smaller population of primordial cells, estimated to be between two and nine (85, 102, 112) of endodermal origin. These cells mitotically divide and migrate to the genital ridges between days 9 to 12 of gestation. In males, the primordial germ cells continue to multiply by mitotic division. At sexual maturity spermatogenesis consists of a population of stem cell spermatogonia, which continue to divide mitotically. This results in constant stem cell renewal, as well as the continual differentiation of a subpopulation of differentiating spermatogonia that are committed to pass through meiosis and the physiological and morphological differentiation associated with spermiogenesis. The duration and characterization of the various stages of spermatogenesis are given in Table 1. In female embryos, primordial germ cells continue

Table 1 Characterization of the spermatogenic stages of the mouse[a]

Stage	Days to fertilization	DNA synthesis	DNA repair
Stem cell spermatogonia	>42	+	+
Differentiating spermatogonia	42–36	+	+
Spermatocytes	35–22	–	+
Early spermatids	21–15	–	+
Late spermatids	14– 8	–	–
Spermatozoa	7– 0	–	–

[a] Adapted from (87,93,113).

to multiply by mitotic division, enter meiosis, and proceed to the oocyte stage. Those oogonia that fail to enter into meiosis degenerate. By the first week after birth, all oocytes attain the dictyate stage in which they remain until the ovulation process is initiated. The differences of the dynamics of gametogenesis in males and females have profound effects for the occurrence of gonadal mosaics: Spermatogenesis proceeds from a stem cell population, capable of cell renewal and clonal expansion even in an adult. By contrast, in females clonal expansion of germ cells is only confined to the short period of mitotic division of primordial germ cells and oogonia during embryonic development.

MOUSE MUTAGENESIS STUDIES

Since the development of the specific locus mutation methods in the mouse (108), the occurrence and nature of spontaneous and induced mutations at six coat-color loci (*a, agouti; b, brown; c, albino; d, dilute; p, pink-eyed dilution; s, piebald*) and one locus controlling the size of the external ear (*se, short-ear*) of the mouse have been intensively studied. Treated or control homozygous wild-type mice are mated to untreated homozygous recessive test-stock mice. Offspring are expected to be heterozygous at all marker loci and to express the wild-type phenotype. In the event of a mutation of the wild-type allele at a marker locus, the mutant offspring will be identified by the characteristic phenotype for the locus involved. Experiments have been carried out with single, acute mutagenic treatments, and the time interval between treatment and fertilization noted. When mutant offspring were recovered, the time interval between treatment and fertilization may be used to determine the gametogenic stage in which a mutation was induced. We may distinguish two types of genetic mosaicism pertinent to such studies: The occurrence of a mutation in the tissues, including the germ cells, of a treated individual is, by definition, a mosaic unless the mutation is eliminated. The second and more interesting

case is the occurrence of fractional mutations in offspring of treated or control mice.

Induced Gonadal Mosaicism

The mutagenic effectiveness of radiation and a number of chemicals in germ cells of the mouse has been studied with the specific locus method (107, 110). The stem cell spermatogonia have been intensively studied since induced mutations in this germ cell stage would be expected to persist in an exposed individual. Thus, the genetic risk would accrue due to the exposure to agents mutagenically active in stem cell spermatogonia during the lifetime of an individual. By contrast, mutations induced in all later spermatogenic stages do not persist but are eliminated as spermatogenesis progresses. Table 2 presents representative specific locus data in stem cell spermatogonia from our laboratory. Radiation, methylnitrosourea (MNU), and ethylnitrosourea (ENU) all increase the mutation rate in stem cell spermatogonia. Ethyl methanesulfonate (EMS) is ineffective in stem cell spermatogonia but effective in inducing mutations in the late spermatid and early spermatozoa stages. If mutations induced in stem cell spermatogonia persist as gonadal mosaics, then the occurrence of repeat mutants recovered in the offspring from a single treated parental male should be expected. The probability of recovering repeat mutants, referred to as clusters, depends upon the fraction of the spermatogonial stem cells carrying the mutation and the number of offspring sampled per treated male. Table 2 also indicates the occurrence of clusters: In the 2 × 80 mg per kg ENU experiment a cluster of two was observed for mutations at the *a* and the *d* loci. In the 160 mg per kg ENU experiment one cluster of two at the *b* locus and two clusters of two each at the *c* and the *d* loci were observed. For the results following 250 mg per kg ENU treatment, three clusters of two mutations each at the *d* locus, two clusters of two mutations each at the *p* locus, and one cluster of two mutations at the *s* locus were recovered. In addition, one cluster of six mutations at the *s* locus and one cluster of two mutations at the *se* locus were recovered in the offspring of untreated control males. A male with more than a single offspring carrying a mutation at a particular locus is assumed to be a gonadal mosaic resulting from a single mutational event. Alternatively, one may consider the observation to be the result of two or more independent mutational events at the same locus in a single parental male. The probability that two independent mutational events at the same locus were recovered from a single male may be calculated as a binomial expansion given the appropriate parameters. This probability is extremely low. Even for the highest mutation rate observed, 16/9,766 (*d* locus; 250 mg per kg ENU) in which 89 offspring were screened per treated parental male, the probability of observing two independent *d* mutations in descendants of the same male is 9×10^{-3}. Thus, the assumption that clustered mutations in offspring of a treated

male represent a single mutational event is probably correct. The fraction of the spermatogonial stem cell population carrying the newly induced mutation may be calculated as twice the frequency of mutant individuals observed in the offspring of a treated male, since the induced mutations are carried as heterozygotes in the affected cells. For the clusters observed in the ENU experiments, the fraction of stem cell spermatogonia carrying the mutations ranges between 4 and 12%. This fraction is quite high and due to the fact that such mutagenic treatments are often associated with cell killing of a portion of the stem cell spermatogonia with subsequent repopulation by clonal expansion of the surviving cells. Indeed, evidence of stem cell spermatogonia killing has been observed as an induced sterile period (15, 20, 32, 81, 94, 95). In the data presented in Table 2 the mean length of the sterile period due to stem cell killing ranged from 9 (EMS) to 107 days (ENU). Based on the assumption that mutant clusters represent the clonal expansion of a single stem cell carrying the induced mutation, the total number of surviving stem cell spermatogonia following mutagenic treatment of parental males in which clusters were observed may be estimated as the reciprocal of the fraction of stem cell spermatogonia carrying the mutation. This value ranges from 8 to 25 cells in the ENU experimental results. For the large cluster observed in the control, it may be assumed that the mutation occurred early in the development of the germ line when approximately five primordial germ cells were present. The results of Carter (13, 14) and Selby (114, 115) should also be noted, in which mice were irradiated in embryonic, newborn, or early postnatal stages and clustering

Table 2 Specific locus mutations in stem cell spermatogonia of the mouse

Group	Offspring	Mutations[b]	Mean sterile period (days)	Cluster events[c]
Control	248 413	22 (16)		1 × 6 (55), 1 × 2 (106)
2 × 3 Gy	18 139	29	33.2	0
MNU 70	21 981	6	24.8	0
ENU 40	11 410	3	18.6	0
ENU 80	13 274	20	23.6	0
ENU 2 × 80	17 665	62 (60)	44.3	2 × 2 (61, 68)
ENU 160	22 332	89 (84)	51.2	5 × 2 (65, 35, 33, 38, 76)
ENU 250	9 766	64 (58)	106.7	6 × 2 (89, 55, 52, 67, 29, 38)
EMS 175	16 809	0	8.8	0
EMS 250	11 372	2	8.8	0

[a] Data sources: Control (28); 2 × 3 Gy (48); MNU (31); ENU 40, 80, 160, 250 (25); ENU 2 × 80 (38); EMS (29); as well as unpublished data from our laboratory.

[b] Independent mutational events given in parentheses

[c] The total number of offspring screened per parental male in matings in which clustered mutational events were observed is given in parentheses.

was observed, presumably due to the occurrence of mutations during the mitotic proliferation phase of germ cell development.

Fractional Mutants

Fractional mutants are gonosomic mosaics, which presumably arise as the result of mutation fixation at an early stage of ontogenesis. Employing the specific locus mutation test methods, fractional mutants may be identified at five (*a, b, c, d, p*) of the seven loci screened. Fractional mutants are identified by the expression of a variegated phenotype representing subpopulations of cells either heterozygous or homozygous recessive at a marker locus. They may be genetically confirmed by the segregation of an induced mutant allele distinguishable from the marker allele at the locus in question or by a distortion in the expected Mendelian segregation when the induced mutant allele is indistinguishable from the recessive marker allele. The occurrence of fractional mutants associated with mutagenic treatment of parental animals implies that the induced DNA adduct is confined to a single strand within the germ cell and is eventually fixed as a mutation following fertilization and mitotic division in the zygote. Russell (102, 103) has reviewed extensive results in the mouse for radiation. Although fractional mutants were observed there was no increase in the frequency of fractional mutants due to irradiation as compared to the frequency in control populations. It may be concluded that the DNA lesions associated with irradiation that are fixed as mutations are not confined to a single strand of DNA. By contrast, chemical mutagens act via the formation of specific DNA adducts that for monofunctional agents should be confined to a single DNA strand. The induction of fractional mouse mutants was recently demonstrated for ENU (37, 39, 75). These observations parallel the results of Auerbach (3) in *Drosophila* in which mutagenic treatment was confined to the post-spermatogonial stages. Thus, the chemically induced DNA adducts occurred in germ cell stages incapable of DNA repair in which the next round of DNA replication takes place after fertilization. Table 3 extends our original observations to include the occurrence in the mouse of specific locus fractional mutations in spermatogonia as well as post-stem cell spermatogonial germ cell stages for mutagenic treatment with MNU, ENU, methyl methanesulfonate (MMS), EMS, and diethyl sulfate (DES). These results indicate the induction of fractional mutants to be mainly confined to the post-spermatogonial germ cell stages in accordance with our previous results and the initial results of Auerbach. The frequency of fractional mutants following mutagenic treatment of post-stem cell spermatogonial stages depends upon the chemical mutagen. ENU-induced mutations in post-stem cell spermatogonial stages are recovered almost exclusively as fractional mutants (eleven fractionals: one whole body). By contrast, the ratio of fractional to whole body mutants in offspring of treated post-stem cell spermatogonial stages for MNU, MMS, EMS and DES is 3:16,

Table 3 Whole-body and fractional specific-locus mutations in the mouse

Group[a]	Germ cell stage[b]	Offspring	Mutations	
			Whole-body	Fractionals
Control		248 413	22 (16)	1
2 × 3 Gy	pg	1 127	1	0
	g	18 139	29	0
MNU 70	pg	16, 207	16	3
	g	21 981	6	0
ENU 40	pg	5 028	0	1
	g	11 410	3	1
ENU 80	pg	4 660	0	0
	g	13 274	20	1
ENU 2 × 80	pg	4 230	1	2
	g	17 665	62 (60)	0
ENU 160	pg	9 958	0	5
	g	22 332	89 (84)	0
ENU 250	pg	3 360	0	3
	g	9 766	64 (58)	1
MMS 20–60	pg	56 206	23	1
EMS 100–250	pg	54 390	16	2
EMS 175, 250	g	28 181	2	0
DES 200, 300	pg	32 614	8	1
DES 200	g	13 551	0	0

[a] Sources of data as given in Table 2 as well as MMS (30); DES (27).
[b] pg = post-stem cell spermatogonia; g = stem cell spermatogonia.

1:23, 2:16, and 1:8, respectively. These differences are probably due to the specificity of DNA adducts induced by the different mutagenic compounds. The frequency of fractional mutants from chemical mutagen treatment of spermatogonia should be noted. The overall frequency of fractional mutants in experimental offspring derived from ENU-treated spermatogonia is 3/74,447, which is ten times the frequency of fractional mutants observed in the historical control of Neuherberg. It appears that MNU is more effective in inducing fractional mutants in differentiating spermatogonia (23 times the control frequency). The mutagens MMS, EMS, and DES are not effective in inducing mutations, fractional or whole-body, in spermatogonia.

Table 4 includes information regarding the mutant alleles recovered as fractional mutants, the time interval between mutagenic treatment and the fertilization event leading to offspring carrying fractional mutants, as well as genetic confirmation results of the presumed fractional mutants. The fractional *d,se* double mutation recovered in the 80 mg per kg ENU treatment group is not likely induced and will not be considered further, since ENU induces almost exclusively point mutations. Of the remaining fractional mutants, most

(14) were recovered within 15 days after mutagenic treatment and represent mutational events due to MNU, ENU, MMS, EMS, or DES treatment. These mutations correspond to the fixation following fertilization of DNA adducts induced in germ cell stages that are incapable of DNA repair and that do not undergo DNA replication (Table 1). Two fractional mutants were recovered between 16 and 27 days following mutagenic treatment with ENU or EMS. The DNA adducts eventually fixed as mutations for these mutants were induced and persisted for a time in germ cell stages capable of DNA repair (Table 1). A final group of four fractional mutants occurred between 37 and 141 days following mutagenic treatment with MNU or ENU. These mutations resulted from DNA adducts that persisted through germ cell stages capable of DNA repair and that survived a number of rounds of DNA replication before eventually being fixed as mutations (Table 1). The observation that fractional mutations may be induced in earlier spermatogenic stages capable of DNA replication and/or DNA repair would imply that the DNA adducts eventually fixed as mutations are not efficiently repaired and do not always template a base-pair substitution at replication. The fraction of the germ cell population in the fractional mutants may estimate the timing of mutation fixation following fertilization. The calculation has been explained in detail (39). Briefly, for induced mutant alleles distinguishable from the recessive marker allele at a locus, the fraction of germ cells carrying the newly induced mutation may be estimated as: $2 \times$ (Offspring carrying newly induced allele/Total offspring). For induced mutant alleles indistinguishable from the recessive marker allele, the calculation of the fraction of germ cells carrying the newly induced allele is calculated as: $2 \times$ ([Homozygous recessive offspring/Total offspring] $- 0.5$) The estimates of the fraction of germ cells carrying the newly induced mutations in fractional mutants ranged from 13 to 107%, with a mean of 49.1%, indicating that the DNA adducts were fixed as mutations during the first round of DNA replication following fertilization and were inherited in one of the two daughter cells after the first cleavage division. Russell (102, 103) came to the same conclusion for the occurrence of spontaneous fractional mutants.

Finally, note that fractional mutants have been recovered in ENU experiments at specific loci in which oocytes were treated (26), as well as at additional genetic endpoints including dominant cataract mutations (39), enzyme electrophoretic variants (75) and enzyme activity mutations (W Pretsch, personal communication).

Mutagenic Treatment of Zygotes

Mutagenesis studies in the mouse were first extended by Generoso to include mutation induction in the early zygote stages (44, 45). The mutagens employed included ENU, EMS, ethylene oxide (EtO), and triethylenemelamine (TEM). Resultant embryos were examined in utero at the 17-day gestation stage. The

Table 4 Specific-locus fractional mutations in the mouse

Group[a]	Allele	Day[b]	Test cross			% Germ cells carrying induced mutations[c]
			Recessive	Wildtype	Mutant allele	
Control	*d,se*	239	25	5	—	—
MNU 70	*c*	9	32 *(c^{ch})*	2	23	81
	a	37	66	9	—	76
	d^{l}	38	7 *(d,se)*	5	4	50
ENU 40	*d^{l}*	1	12 *(d,se)*	1	15	107
	p^{d}	141	33	24	5	16
ENU 80	*d,se*	117	66 *(d,se)*	9	—	—
ENU 2 × 80	*p^{d}*	1	34 *(p)*	19	12	37
	p	27	61	19	—	53
ENU 160	*p*	2	51	29	—	28
	p	10	47	10	—	65
	p	2	91	29		52
	c	12	22 *(c^{ch})*	3	20	88
	d	15	31 *(d,se)*	34	9	24
ENU 250	*p^{d}*	1	51 *(p)*	17	21	47
	d	1	18 *(d,se)*	12	2	13
	p	3	26	9	—	49
	d^{l}	87	15 *(d,se)*	9	6	40
MMS 60	*d^{l}*	15	13 *(d,se)*	16	3	19
EMS 100	*c*	14	60 *(c^{ch})*	43	19	31
EMS 175	*d^{l}*	16	25 *(d,se)*	5	12	57
DES 200	*p^{d}*	5	15 *(p)*	13	9	49

[a] Sources of data as in Tables 2 and 3.

[b] Interval between treatment and conception. See Table 1 for the duration of the spermatogenic stages.

[c] For induced mutant alleles distinguishable from the recessive marker allele, calculated as: 2 × (Offspring carrying newly induced allele/Total offspring) For induced mutant alleles indistinguishable from the recessive marker allele, calculated as: 2 × ([Homozygous recessive offspring/Total offspring] −0.5)

mutagenic effects observed ranged from death of conceptuses prior to or around the time of implantation, misdifferentiation in later developmental stages incompatible with embryo survival, and developmental abnormalities in the surviving embryos. The spectrum of mutagenic effects observed was mutagen-specific. These experiments identified the early zygotic stages to be extremely sensitive to the effects of mutagenic agents, with profound implications for the human situation. Further, the concept of teratological effects of chemical mutagens acting via the fixation of induced mutations in very early developmental stages was proposed.

Russell, Bangham, Stelzner, et al (104) employed the mouse-specific locus method to determine if the early zygotic stages of the mouse were also sensitive

to the induction of coat color mutations by ENU. Indeed, a high frequency of recessive specific-locus mutations was observed. In comparison to the observed mutation rate following ENU treatment of stem cell spermatogonia, the mutation rate observed for ENU treatment 2.5-3 h post-mating was approximately ten times higher. The mutations recovered occurred mainly as fractional mutants (eight fractionals: three whole body). Genetic confirmation tests demonstrated germ-line involvement and the average fraction of the germ line carrying the mutation was calculated to be approximately 50%. Thus, as for the occurrence of fractional mutants following treatment in the parental germ cells, treatment of early zygotic stages with ENU results in DNA adducts confined to a single DNA strand, which are fixed as a mutation at the next round of DNA replication.

Induced Mutations in Somatic Cells

Russell & Major (105) first developed an experimental protocol to screen for induced mutations in the somatic cells of treated embryos heterozygous at coat pigmentation loci. Although the presumed somatic mutations cannot be genetically confirmed, the pigmentation spots presumed to represent the clonal expansion of mutations induced in the melanoblasts have been classified as genetically relevant, based upon the coloration and position of the spot on the body (33, 105). Fahrig & Neuhäuser-Klaus (36) have examined the hairs taken from genetically relevant spots and confirmed that such hairs are phenotypically identical with hairs from mice of the genotype suspected at the pigmentation spot.

The frequency and size of the induced pigmentation spots follows the expectations for the dynamics of mutation induction in a mitotically expanding target cell population (34, 106). For mutagenic treatment in earlier embryonic stages, the frequency of spots is reduced, but the average size of the induced spots is large. When the mutagenic treatment is applied at later embryonic stages, the frequency of mutant spots is increased and the average size of the pigmentation spots is decreased.

SPONTANEOUS GENETIC MOSAICISM IN THE MOUSE

Apart from the carefully controlled mutation studies at defined mouse pigmentation loci with mutagenic treatments as outlined above, fractional mutants may and do occur spontaneously. Although the spontaneous occurrence of fractional mutants is a rare event, two interesting situations are considered in which the frequency of fractional mutants is relatively high: the occurrence of apparent reverse mutations in mice homozygous for genetically unstable mutant alleles, and the loss of heterozygosity in mice resulting in the expression of the recessive phenotype in affected cells.

Unstable Alleles

A number of forward mutations in the mouse have been demonstrated to be genetically unstable (82, 102, 109, 120). The reversion events may occur at different times between gametogenesis in parental mice through the developmental period of an individual leading to reverse mutations recovered as whole body mutants, gonosomic mosaics, or somatic mutation events.

The *a* allele at the *agouti* locus has recently been molecularly characterized and shown to be due to an 11-kb insert in the first intron of the *agouti* gene resulting in a null allele (10, 83). The *a* allele is capable of mutating to a number of alternative alleles including a^t, A^y, and A^w, in which there is partial or full restoration of gene function at the *agouti* locus. It is proposed that the reversion events are associated with alteration in the structure of the DNA insertion resulting in the *a* allele. Indeed, Bultman, Michaud & Woychik (10) have shown for an a^t mutation recovered in offspring of *a/a* homozygotes that the DNA insert within the first intron is shortened to 5.5 kb. This particular mutational event as well as a number of additional mutational events at the *agouti* locus recovered in offspring of *a/a* parental mice occurred as whole-body mutants due to a mutation fixed in the parental germ cells or a very early stage in development of the mutant individual (50, 109). However, fractional mutants in mice of the expected genotype *a/a* have also been recovered (50, 101) and genetically confirmed, indicating that the reversion events may occur during mitosis in developmental stages of an individual. We have also recovered a gonosomic mosaic mouse with patches of cells of the genotype A^y/a or *a/a* in inbred strain C57BL/6 (normally *a/a*). Test crosses indicated the involvement of the germ line and estimated 16% of the germ cells to carry the A^y mutation; 3 A^y/a and 35 *a/a* offspring were recovered in the cross of the mosaic to *a/a* (J Favor, unpublished data).

The d^v allele at the *dilute* locus carried by a number of standard strains of the laboratory mouse including strain DBA/2 is due to the insertion of an ecotropic-specific retroviral sequence (*Emv-3*) in the vicinity of the *dilute* locus (65). Proviral sequences are known to excise, and reversion of the d^v allele is associated with excision of the *Emv-3* insertion (18, 59, 65). Most reversion events have been reported as germ-line excision events resulting in whole-body mutants. However, Seperack, Strobel, Corrow, et al (116) have demonstrated a gonosomic mosaic. Excision of the proviral sequence results in the loss of all but a single LTR and is proposed to be due to intrachromosomal recombination between the proviral LTRs (18, 59, 116). Recombination may be more likely to occur in meiotic cells, which would explain the higher frequency of germ-line revertants as compared to reversions in the somatic cells.

The p^{un} allele at the *pink-eyed dilution* locus is the most unstable allele identified in the mouse that results in an observable phenotype. Reversion

often occurs and mutants are recovered as whole-body mutants or gonosomic mosaics (82, 111). Recent studies have shown the p^{un} allele to be due to a large (>80 kb) tandem duplication (7, 8, 47), and reversion has been associated with loss of the duplicated sequences. Although flanking markers were not studied, the authors propose the mechanism of reversion to be unequal crossing over analogous to the Bar eye mutation of *Drosophila* (121).

The *pe* allele at the *pearl* locus of the mouse is also unstable with a high frequency of reversion. Although the exact nature of the mutation is not yet known, the high rate of reversion of the *pe* allele has led Whitney & Lamoreux (124) to suggest that the forward mutation to *pe* may be the result of an insertion of a transposable element. Russell (102) has characterized reversion events in *pe/pe* homozygous mice. Both presumed somatic and gonosomic mutants have been recovered. The fraction of the coat expressing the pe^+/pe phenotype in recovered mutants was shown to vary between 0.1 and 100%. The involvement of the germ line was tested by genetic confirmation crosses. Many individuals with very low numbers of somatic cells expressing the pe^+/pe phenotype were not gonadal mosaic for the reverse mutation, indicating that the reversion took place at a later stage in embryonic development. At the other extreme, individuals expressing the pe^+/pe coat color phenotype in almost 100% of the coat transmitted the pe^+ allele at a high frequency. However, the results indicated that the individuals were gonadal mosaics for the pe^+ mutation and the reverse mutation must have occurred at a very early stage in development before the divergence of the germinal and somatic cell lineages. Few intermediate phenotypes were recovered, suggesting that reversion events took place either very early in development at approximately the two-cell stage zygote or at about the 10-day post-conception embryo. No whole-body pe^+/pe heterozygotes indicative of reversion in the parental germ cells were reported.

The cukaryotic genome including the mouse (22) is replete with amplified short nucleotide repeat sequences (53, 63, 118). Such sequences, referred to as minisatellites or variable number tandem repeats, consist of short base-pair elements tandemly duplicated to form reiterated arrays up to 20 kb long (56). To date minisatellites have not been associated with mutation of genes but normally reside in noncoding DNA sequences of the genome. Characterizing mice at the DNA level it has been shown that some sequences, referred to as hypervariable regions, exhibit high somatic and germinal mutation rates. Kelly, Bulfield, Collick, et al (66) have estimated the germinal and somatic mutation rate at the *Ms6-hm* allele on mouse chromosome 4 to be 2.5 and 2.8%, respectively. Three mosaic mutants were genetically tested and germ-line involvement was demonstrated. The percentage of somatic cells carrying the new mutations ranged from 15 to 40%, with approximately 5 to 40% germ-line involvement. Dallas (19) has estimated the germ-line mutation rates at three loci (*Ckmm, Cyp1a2,* and *Gfap*) to be between 10^{-2} and 10^{-4}. Finally, in

transmission studies of parental and offspring mice, Dubrova, Jeffreys & Malashenko (23) have estimated the germ-line mutation rate at the *Hm-2* locus to be 8.5×10^{-3}. Gonadal mosaicism in parental mice was inferred from clustered mutations recovered as repeats in offspring of a single mating in which the somatic cells of the parental mice were not affected. All of the above studies indicate the mutation rates in germ cells at hypervariable regions to be approximately three orders of magnitude higher than the mutation rates observed at visible loci in the mouse. Gibbs, Collick, Kelly & Jeffreys (46) have determined more precisely the timing of the mutational events at the *Hm-2* locus. They have confirmed the high germinal (3.5%) and somatic mutation rates and demonstrated the occurrence of gonosomic mosaic mutants. Further, by examining embryonic and extraembryonic tissues of early developmental stages they have determined that a significant proportion of the *Hm-2* mutations arise within the first few cleavage divisions following fertilization.

Mitotic Crossing Over

The generation of mouse mosaics with patches expressing the recessive phenotype in normally heterozygous individuals may be due to a number of mechanisms including mutation of the dominant allele, gene conversion, chromosomal nondisjunction, and mitotic recombination. Additional data are required to distinguish among the proposed mechanisms. Mitotic recombination has been implied in a number of studies (12, 43, 50, 98). A case for mitotic crossing over may be made if the reciprocal crossover products can be identified. In fact, twin spots in heterozygous mice have been identified, presumably representing adjacent clones from the daughter cells following a mitotic recombination event in which the alternative homozygous cell types are phenotypically distinguishable from each other as well as from the heterozygous state. Russell (102) and Bateman (6) have both observed twin spotting of the sort *c/c*; c^{ch}/c^{ch}, which arose in c/c^{ch} heterozygotes. Fahrig (35) has reported mosaic mice with twin spots of the genotype $p\text{-}c^{ch}/p\text{-}c^{ch}$; +-+/+-+, which arose in $p\text{-}c^{ch}$/+-+ animals. Fisher, Stephenson & West (40) have recovered a mouse with the twin spot *p-+/p-+; +-c/+-c* in a mouse of the genotype *p-+/+-c*. In all cases mitotic recombination between the marker alleles and the centromere could explain the observations. The authors have estimated the occurrence of twin spotting to be low. Other, more complicated events could also explain the generation of twin spots in these studies. A definitive proof of the occurrence of mitotic recombination would require genotyping of the affected cells, which now may be possible with the use of sensitive molecular techniques.

HUMAN GENETIC STUDIES

Recently, there has been a virtual explosion in the literature of documented cases of genetic mosaicism in humans. Although confirmation of presumed

genetic mosaicism in earlier studies was restricted by low kindred numbers and often a lack of informative genotypes for segregation analyses, recent studies have profited from molecular techniques, which allow direct identification of the mutant alleles and their segregation. The generation and occurrence of genetic mosaicism in humans closely parallels the situation in the mouse. Although an extensive survey of the human genetic literature on mosaicism goes beyond the scope of this review, some major aspects should be discussed briefly.

Gonosomic Mosaicism

In extensive reviews, Hall (52) and Mohrenweiser (86) have noted that a substantial portion of newly occurring mutations in humans arise as gonadal mosaics. These observations raise the question whether mitotic cells are more sensitive than meiotic cells to the occurrence of mutation. Further, such findings have profound implications on genetic counseling (11, 52, 61, 89, 125), where a recurrence risk exists in subsequent sibs of an apparent de novo mutant proband if the mutation is present as a gonadal mosaic in a parent. This situation is most extreme for the observation of recurrence of dominant lethal mutations among sibs of gonadal mosaic, unaffected parents (16, 54, 55). Finally, upon identification of suspect mosaicism in parents due to the recurrence of de novo mutations in offspring, a careful examination at the phenotypic or molecular level may identify somatic mosaicism as a less severe phenotype or a portion of cells carrying the mutant alleles in a parent (5, 17, 49, 51, 52, 123, 126). Identification of identical mutations at the DNA level transmitted to more than a single offspring from unaffected parents in a family also provides evidence for the existence of parental gonadal mosaicism (4, 5, 49, 72, 123).

Unstable Alleles

There are interesting data indicating the existence of potentially unstable alleles in humans. Partial gene duplications have been demonstrated for a number of germ-line mutations (21, 57, 58, 73, 74, 79, 88, 91) and reversion has been demonstrated (128). A germ-line mutation at the neurofibromatosis type I locus is due to the insertion of a transposable *Alu* sequence within an intron (122) and this mutation could conceivably be unstable via excision of the *Alu* insertion. Rare patches of presumably revertant cells in Duchenne muscular dystrophy patients are due to second-site mutations (67). The occurrence of such "revertants" must be dependent on the original forward mutation and whether it is capable of restoration by a second-site mutation. The existence of hypervariable minisatellite regions in human DNA is also well documented (62, 63, 92) and high gonadal (64) and somatic (2) mutation rates have been determined. Finally, Müller & Scott (90) have reviewed the human genetic literature for those hereditary conditions in which loss of heterozygosity in somatic cells

via chromosomal nondisjunction, mutation, gene conversion, or mitotic recombination may be important.

One of the most remarkable recent discoveries by far has been the association of mutations with amplified trinucleotide repeat sequences. To date six diseases have been associated with unstable expansion of trinucleotide repeats; fragile X (41, 68), spinal and bulbar muscular atrophy (70), myotonic dystrophy (9), Huntington's disease (24), spinocerebellar ataxia 1 (96), and dentatorubral-pallidoluysian atrophy (69). In all cases these diseases show a relatively high mutation rate as well as earlier age of onset and increased severity of the phenotype in successive generations as the amplified sequences expand. The instability of the amplified sequences has been studied in both germ-line and somatic cells (1, 41, 60, 69, 71, 78, 96, 100, 127, 129, 130), and there are differences in the relative somatic and germ-line instability of the different diseases. The mouse homolog of the Huntington disease gene has recently been identified. The trinucleotide sequence associated with expansion was shown to be conserved and as in humans amplified (75a).

CONCLUSIONS

This review has documented and clarified the mechanisms involved in the occurrence of genetic mosaicism in the mouse. As new methodologies become available to study these phenomena, e.g. DNA methods when an involved gene has been cloned, our understanding of the mechanisms will increase. We may anticipate a more accurate documentation of the occurrence of genetic mosaicism and probably the identification of mechanisms to explain the occurrence of mosaicism dependent upon particular loci or alleles. The parallels between mouse and man concerning genetic mosaics are striking. Mouse homologs of human genes associated with amplified trinucleotide repeat sequences have been identified, and we may foresee an intensive study of this phenomenon in the mouse. We would like to close with an apology. We have relied mostly upon specific locus mutation results from Neuherberg so that we could incorporate previously unpublished data. Clustered mutations indicative of gonadal mosaicism also have been reported from Oak Ridge and MRC-Harwell. Finally, we have limited the scope of this review to genetic mosaicism due to mutational events. The exciting areas of research concerning functional mosaicism (76) and the production and study of chimeras (97) had to be totally excluded from consideration.

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Literature Cited

1a. Allen JW, Bridges BA, Lyon MF, Montrose MJ, Russell LB, eds. 1990. *Banbury Report 34: Biology of Mammalian Germ Cell Mutagenesis.* Cold Spring Harbor: Cold Spring Harbor Press. 461 pp.

1. Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, et al. 1993. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nature Genet.* 4:398–403
2. Armour JAL, Patel I, Thein SL, Fey MF, Jeffreys, AJ. 1989. Analysis of somatic mutations at human minisatellite loci in tumors and cell lines. *Genomics* 4:328–34
3. Auerbach C. 1947. The induction by mustard gas of chromosomal instabilities in *Drosophila melanogaster. Proc. R. Soc. Edinburgh, Sect. B* 62:307–20
4. Bakker E, Van Broeckhoven C, Bonten EJ, van de Vooren MJ, Veenema H, et al. 1987. Germline mosaicism and Duchenne muscular dystrophy mutations. *Nature* 329:554–56
5. Bakker E, Veenema H, Den Dunnen JT, Van Broeckhoven C, Grootscholten PM, et al. 1989. Germinal mosaicism increases the recurrence risk for "new" Duchenne muscular dystrophy mutations. *J. Med. Genet.* 26:553–59
6. Bateman AJ. 1967. A probable case of mitotic crossing-over in the mouse. *Genet. Res.* 9:375–76
7. Brilliant MH, Gondo Y. 1992. Molecular characterization of the p^{un} allele of the mouse pink-eyed dilution locus. *Pigment Cell Res.* 5:271–73
8. Brilliant MH, Gondo Y, Eicher EM. 1991. Direct molecular identification of the mouse pink-eyed unstable mutation by genome scanning. *Science* 252:566–69
9. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, et al. 1992. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell* 68:799–808
10. Bultman SJ, Michaud EJ, Woychik RP. 1992. Molecular characterization of the mouse agouti locus. *Cell* 71:1195–204
11. Carlson EA, Desnick RJ. 1979. Mutational mosaicism and genetic counseling in retinoblastoma. *Am. J. Med. Gen.* 4:365–81
12. Carter TC. 1952. A mosaic mouse with an anomalous segregation ratio. *J. Genet.* 51:1–6
13. Carter TC. 1960. Mutation induced in germ cells of the foetal female mouse. *Genet. Res.* 1:59–61
14. Carter TC, Lyon MF, Phillips RJS. 1960. The genetic sensitivity to X-rays of mouse foetal gonads. *Genet. Res.* 1:351–55
15. Cattanach BM. 1974. Spermatogonial stem cell killing in the mouse following single and fractionated X-ray doses, as assessed by length of sterile period. *Mutat. Res.* 25:53–62
16. Cohn DH, Starman BJ, Blumberg B, Byers PH. 1990. Recurrence of lethal *osteogenesis imperfecta* due to parental mosaicism for a dominant mutation in a human type I collagen gene (COLIA1). *Am. J. Hum. Genet.* 46:591–601
17. Constantinou-Deltas CD, Ladda RL, Prockop DJ. 1993. Somatic cell mosaicism: Another source of phenotypic heterogeneity in nuclear families with osteogenesis imperfecta. *Am. J. Med. Genet.* 45:246–51
18. Copeland NG, Hutchison KW, Jenkins NA. 1983. Excision of the DBA ecotropic provirus in dilute coat-color revertants of mice occurs by homologous recombination involving the viral LTRs. *Cell* 33:379–87
19. Dallas JF. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. *Mamm. Genome* 3:452–56
20. De Rooij DG, Kramer MF. 1968. Spermatogonial stem cell renewal in the rat, mouse and golden hamster. A study with

the alkylating agent myleran. *Z. Zellforsch.* 92:400–5
21. Devlin RH, Deeb S, Brunzell J, Hayden MR. 1990. Partial gene duplication involving exon-Alu interchange results in lipoprotein lipase deficiency. *Am. J. Hum. Genet.* 45:112–19
22. Dietrich W, Katz H, Lincoln SE, Shin H-S, Friedman J, et al. 1992. A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131:423–47
23. Dubrova YE, Jeffreys AJ, Malashenko AM. 1993. Mouse minisatellite mutations induced by ionizing radiation. *Nat. Genet.* 5:92–94
24. Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, et al. 1993. Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nat. Genet.* 4:387–92
25. Ehling UH, Neuhäuser-Klaus A. 1984. Dose-effect relationships of germ-cell mutations in mice. In *Problems of Threshold in Chemical Mutagenesis,* ed. Y Tazima, S Kondo, Y Kuroda, pp. 15–25. Tokyo: Kokusai-bunken. 190 pp.
26. Ehling UH, Neuhäuser-Klaus A. 1988. Induction of specific-locus mutations in female mice by 1-ethyl-1-nitrosourea and procarbazine. *Mutat. Res.* 202:139–46
27. Ehling UH, Neuhäuser-Klaus A. 1988. Induction of specific-locus and dominant-lethal mutations in male mice by diethyl sulfate (DES). *Mutat. Res.* 199: 191–98
28. Ehling UH, Neuhäuser-Klaus A. 1988. Induction of specific-locus and dominant-lethal mutations by cyclophosphamide and combined cyclophosphamide-radiation treatment in male mice. *Mutat. Res.* 199:21–30
29. Ehling UH, Neuhäuser-Klaus A. 1989. Induction of specific-locus mutations in male mice by ethyl methanesulfonate (EMS). *Mutat. Res.* 227:91–95
30. Ehling UH, Neuhäuser-Klaus A. 1990. Induction of specific-locus and dominant lethal mutations in male mice in the low dose range by methyl methanesulfonate (MMS). *Mutat. Res.* 230:61–70
31. Ehling UH, Neuhäuser-Klaus A. 1991. Induction of specific-locus and dominant lethal mutations in male mice by 1-methyl-1-nitrosourea (MNU). *Mutat. Res.* 250:447–56
32. Erickson BH, Hall GG. 1983. Comparison of stem-spermatogonial renewal and mitotic activity in the γ-irradiated mouse and rat. *Mutat. Res.* 108:317–35
33. Fahrig R. 1975. A mammalian spot test: Induction of genetic alterations in pigment cells of mouse embryos with X-rays and chemical mutagens. *Mol. Gen. Genet.* 138:309–14
34. Fahrig R. 1978. The mammalian spot test: A sensitive in vivo method for the detection of alterations in somatic cells of mice. In *Chemical Mutagens: Principles and Methods for their Detection,* ed. A Hollaender, FJ de Serres, 5:151–76. New York/London: Plenum. 348 pp.
35. Fahrig R. 1984. Genetic mode of action of cocarcinogens and tumor promoters in yeast and mice. *Mol. Gen. Genet.* 194:7–14
36. Fahrig R, Neuhäuser-Klaus A. 1985. Similar pigmentation characteristics in the specific-locus and the mammalian spot test. *J. Hered.* 76:421–26
37. Favor J. 1990. Toward an understanding of the mechanisms of mutation induction by ethylnitrosourea in germ cells of the mouse. See Ref. 1a, pp. 221–35
38. Favor J, Neuhäuser-Klaus A, Ehling UH. 1988. The effect of dose fractionation on the frequency of ethylnitrosourea-induced dominant cataract and recessive specific locus mutations in germ cells of the mouse. *Mutat. Res.* 198:269–75
39. Favor J, Neuhäuser-Klaus A, Ehling UH. 1990. The frequency of dominant cataract and recessive specific-locus mutations and mutation mosaics in F_1 mice derived from post-spermatogonial treatment with ethylnitrosourea. *Mutat. Res.* 229:105–14
40. Fisher G, Stephenson DA, West JD. 1986. Investigation of the potential for mitotic recombination in the mouse. *Mutat. Res.* 164:381–88
41. Fu Y-H, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, et al. 1991. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 67:1047–58
42. Gardner RL. 1968. Mouse chimaeras obtained by the injection of cells into the blastocyst. *Nature* 220:596–97
43. Geissler EN, McFarland EC, Russell ES. 1981. Analysis of pleiotropism at the dominant white-spotting (*W*) locus of the house mouse: A description of ten new *W* alleles. *Genetics* 97:337–61
44. Generoso WM, Rutledge JC, Cain KT, Hughes LA, Braden PW. 1987. Exposure of female mice to ethylene oxide within hours after mating leads to fetal malformation and death. *Mutat. Res.* 176:269–74
45. Generoso WM, Rutledge JC, Cain KT, Hughes LA, Downing DJ. 1988. Muta-

gen-induced fetal anomalies and death following treatment of females within hours after mating. *Mutat. Res.* 199:175–81

46. Gibbs M, Collick A, Kelly RG, Jeffreys AJ. 1993. A tetranucleotide repeat mouse minisatellite displaying substantial somatic instability during early preimplantation development. *Genomics* 17:121–28
47. Gondo Y, Gardner JM, Nakatsu Y, Durham-Pierre D, Deveau S, et al. 1993. High-frequency genetic reversion mediated by a DNA duplication: The mouse pink-eyed unstable mutation. *Proc. Natl. Acad. Sci. USA* 90:297–301
48. Graw J, Favor J, Neuhäuser-Klaus A, Ehling UH. 1986. Dominant cataract and recessive specific locus mutations in offspring of X-irradiated male mice. *Mutat. Res.* 159:47–54
49. Greger V, Passarge E, Horsthemke B. 1990. Somatic mosaicism in a patient with bilateral retinoblastoma. *Am. J. Hum. Genet.* 46:1187–93
50. Grüneberg H. 1966. The case for somatic crossing over in the mouse. *Genet. Res.* 7:58–75
51. Hall J. 1990. Somatic and germ-line mosaicism in autosomal dominant antecubital pterygium. *Clin. Genet.* 37:160
52. Hall JG. 1988. Review and hypotheses: Somatic mosaicism: Observations related to clinical genetics. *Am. J. Hum. Genet.* 43:355–63
53. Hamada H, Petrino MG, Takunaga T. 1982. A novel repeated element with z-DNA-forming potential is widely found in evolutionary diverse eukaryotic genomes. *Proc. Natl. Acad. Sci. USA* 79:6465–69
54. Happle R. 1986. The McCune-Albright syndrome: a lethal gene surviving by mosaicism. *Clin. Genet.* 29:321–24
55. Happle R. 1987. Lethal genes surviving by mosaicism: A possible explanation for sporadic birth defects involving the skin. *J. Am. Acad. Derm.* 16:899–906
56. Hearne CM, Ghosh S, Todd JA. 1992. Microsatellites for linkage analysis of genetic traits. *Trends Genet.* 8:288–94
57. Hu X, Burghes AHM, Ray PN, Thompson MW, Murphy EG, Worton RG. 1988. Partial gene duplication in Duchenne and Becker muscular dystrophies. *J. Med. Genet.* 25:369–76
58. Hu X, Ray PN, Worton RG. 1991. Mechanisms of tandem duplication in the Duchenne muscular dystrophy gene include both homologous and nonhomologous intrachromosomal recombination. *EMBO J.* 10:2471–77
59. Hutchison KW, Copeland NG, Jenkins NA. 1984. Dilute-coat-color locus of mice: Nucleotide sequence analysis of the d^{+2J} and d^{+Ha} revertant alleles. *Mol. Cell. Biol.* 4:2899–904
60. Imbert G, Kretz C, Johnson K, Mandel J-L. 1993. Origin of the expansion mutation in myotonic dystrophy. *Nat. Genet.* 4:72–76
61. Jeanpierre M. 1992. Germinal mosaicism and risk calculation in X-linked diseases. *Am. J. Hum. Genet.* 50:960–67
62. Jeffreys AJ, Neumann R, Wilson V. 1990. Repeat unit sequcnce variation in minisatellites: A novel source of DNA polymorphism for studying variation and mutation by single molecule analysis. *Cell* 60:473–85
63. Jeffreys AJ, Wilson V, Thein SL. 1985. Hypervariable "minisatellite" regions in human DNA. *Nature* 314:67–73
64. Jeffreys AJ, Royle NJ, Wilson V, Wong Z. 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature* 332:278–81
65. Jenkins NA, Copeland NG, Taylor BA, Lee BK. 1981. Dilute (*d*) coat colour mutation of DBA/2J mice is associated with the site of integration of an ecotropic MuLV genome. *Nature* 293: 370–74
66. Kelly R, Bulfield G, Collick A, Gibbs M, Jeffreys AJ. 1989. Characterization of a highly unstable mouse minisatellite locus: Evidence for somatic mutation during early development. *Genomics* 5:844–56
67. Klein CJ, Coovert DD, Bulman DE, Ray PN, Mendell JR, Burghes AHM. 1992. Somatic reversion/suppression in Duchenne muscular dystrophy (DMD): Evidence supporting a frame-restoring mechanism in rare dystrophin-positive fibers. *Am. J. Hum. Genet.* 50:950–59
68. Knight SJL, Flannery AV, Hirst MC, Campbell L, Christodoulou Z, et al. 1993. Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell* 74:127–34
69. Koide R, Ikeuchi T, Onodera O, Tanaka H, Igarashi S, et al. 1994. Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat. Genet.* 6:9–13
70. LaSpada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck H. 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 352:77–79
71. Lavedan C, Hofmann-Radvanyi H, Shelbourne P, Rabes J-P, Duros C, et al. 1993. Myotonic dystrophy: Size- and

sex-dependent dynamics of CTG meiotic instability, and somatic mosaicism. *Am. J. Hum. Genet.* 52:875–83

72. Lebo RV, Olney RK, Golbus MS. 1990. Somatic mosaicism at the Duchenne locus. *Am. J. Med. Genet.* 37: 187–90
73. Lehrman MA, Goldstein JL, Russell DW, Brown MS. 1987. Duplication of seven exons in LDL receptor gene caused by Alu-Alu recombination in a subject with familial hypercholesterolemia. *Cell* 48:827–35
74. Lelli N, Ghisellini M, Calandra S, Gaddi A, Ciarrocchi A, et al. 1991. Duplication of exons 13, 14 and 15 of the LDL-receptor gene in a patient with heterozygous familial hypercholesterolemia. *Hum. Genet.* 86:359–62
75. Lewis SE, Barnett LB, Popp RA. 1990. Mosaic mutants induced by ethylnitrosourea in late germ cells of mice. See Ref. 1a, pp. 237–45

75a. Lin B, Nasir J, MacDonald H, Hutchinson G, Graham RK, et al. 1994. Sequence of the murine Huntington disease gene: evidence for conservation, and polymorphism in a triplet (CCG) repeat alternate splicing. *Hum. Mol. Genet.* 3: 85–92

76. Lyon MF. 1992. Some milestones in the history of X-chromosome inactivation. *Annu. Rev. Genet.* 26:17–28
77. Lyon MF, Searle AG, eds. 1989. *Genetic Variants and Strains of the Laboratory Mouse.* Oxford/New York/Tokyo: Oxford Univ. Press. 876 pp. 2nd. ed.
78. MacDonald ME, Barnes G, Srinidhi J, Duyao MP, Ambrose CM, et al. 1993. Gametic but not somatic instability of CAG repeat length in Huntington's disease. *J. Med. Genet.* 30:982–86
79. Maeda N, Yang F, Barnett DR, Bowman BH, Smithies O. 1984. Duplication within the haptoglobin Hp^2 gene. *Nature* 309:131–35
80. Markert CL, Petters RM. 1978. Manufactured hexaparental mice show that adults are derived from three embryonic cells. *Science* 202:56–58
81. Meistrich ML, Hunter NR, Suzuki N, Trostle PK, Withers HR. 1978. Gradual regeneration of mouse testicular stem cells after exposure to ionizing radiation. *Radiat. Res.* 74:349–62
82. Melvold RW. 1971. Spontaneous somatic reversion in mice: Effects of parental genotype on stability at the *p*-locus. *Mutat. Res.* 12:171–74
83. Miller MW, Duhl DMJ, Vrieling H, Cordes SP, Ollman MK, et al. 1993. Cloning of the mouse *agouti* gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. *Genes Dev.* 7:454–67
84. Mintz B. 1967. Gene control of mammalian pigmentary differentiation, I. Clonal origin of melanocytes. *Proc. Natl. Acad. Sci. USA* 58:344–51
85. Mintz B. 1974. Gene control of mammalian differentiation. *Annu. Rev. Genet.* 8:411–70
86. Mohrenweiser H. 1994. Impact of the molecular spectrum of mutational lesions on estimates of germinal gene-mutation rates. *Mutat. Res.* 304:119–37
87. Monesi V. 1962. Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. *J. Cell Biol.* 14:1–18
88. Monnat RJ Jr, Chiaverotti TA, Hackmann AFM, Maresh GA. 1992. Molecular structure and genetic stability of human hypoxanthine phosphoribosyltransferase (HPRT) gene duplications. *Genomics* 13:788–96
89. Moss C, Burn J. 1988. Genetic counselling in hypomelanosis of Ito: case report and review. *Clin. Genet.* 34:109–15
90. Müller Hj, Scott R. 1992. Hereditary conditions in which the loss of heterozygosity may be important. *Mutat. Res.* 284:15–24
91. Murru S, Casula L, Pecorara M, Mori P, Cao A, Pirastu M. 1990. Illegitimate recombination produced a duplication within the FVIII gene in a patient with mild hemophilia A. *Genomics* 7:115–18
92. Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, et al. 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* 235:1616–22
93. Oakberg EF. 1956. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. *Am. J. Anat.* 99:507–16
94. Oakberg EF. 1975. Effects of radiation on the testis. In *Handbook of Physiology, Sect. 7, Endocrinology* 5:233–43. Baltimore, MD: Williams & Wilkins
95. Oakberg EF. 1978. Differential spermatogonial stem-cell survival and mutation frequency. *Mutat. Res.* 50:327–40
96. Orr HT, Chung M-Y, Banfi S, Kwiatkowski TJ Jr, Servadio A, et al. 1993. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat. Genet.* 4:221–26
97. Palmiter RD, Brinster RL. 1986. Germline transformation of mice. *Annu. Rev. Genet.* 20:465–99
98. Panthier J-J, Guénet J-L, Condamine H, Jacob F. 1990. Evidence for mitotic

recombination in $W^{ei}/+$ heterozygous mice. *Genetics* 125:175–82
99. Rawles ME. 1947. Origin of pigment cells from the neural crest in the mouse embryo. *Physiol. Zool.* 20:248–66
100. Read AP. 1993. Huntington's disease: testing the test. *Nat. Genet.* 4:329–30
101. Robinson R. 1957. Mosaicism in mammals. *Genetica* 29:120–45
102. Russell LB. 1964. Genetic and functional mosaicism in the mouse. In *The Role of Chromosomes in Development*, ed. M Locke, pp. 153–81. New York/London: Academic. 290 pp.
103. Russell LB. 1979. Analysis of the albino-locus region of the mouse. II. Mosaic mutants. *Genetics* 91:141–47
104. Russell LB, Bangham JW, Stelzner KF, Hunsicker PR. 1988. High frequency of mosaic mutants produced by N-ethyl-N-nitrosourea exposure of mouse zygotes. *Proc. Natl. Acad. Sci. USA* 85:9167–70
105. Russell LB, Major MH. 1957. Radiation-induced presumed somatic mutations in the house mouse. *Genetics* 42: 161–75
106. Russell LB, Montgomery CS. 1982. Supermutagenicity of ethylnitrosourea in the mouse spot test: Comparisons with methylnitrosourea and ethylnitrosourea. *Mutat. Res.* 92:193–204
107. Russell LB, Selby PB, von Halle E, Sheridan W, Valcovic L. 1981. The mouse specific-locus test with agents other than radiations. Interpretation of data and recommendations for future work. *Mutat. Res.* 86:329–54
108. Russell WL. 1951. X-ray-induced mutations in mice. *Cold Spring Harbor Symp. Quant. Biol.* 16:327–36
109. Schlager G, Dickie MM. 1971. Natural mutation rates in the house mouse: Estimates for five specific loci and dominant mutations. *Mutat. Res.* 11: 89–96
110. Searle AG. 1974. Mutation induction in mice. In *Advances in Radiation Biology*, ed. JT Lett, H Adler, M Zelle, 4:131–207. London/New York: Academic. 435 pp.
111. Searle AG. 1977. The use of pigment loci for detecting reverse mutations in somatic cells of mice. *Arch. Toxicol.* 38:105–8
112. Searle AG. 1978. Evidence from mutable genes concerning the origin of the germ line. In *Genetic Mosaics and Chimeras in Mammals*, ed. LB Russell, 12:209–24. New York/London: Plenum. 485 pp.
113. Sega GA. 1974. Unscheduled DNA synthesis in the germ cells of male mice exposed in vivo to the chemical mutagen ethyl methanesulfonate. *Proc. Natl. Acad. Sci. USA* 71:4955–59
114. Selby PB. 1973. X-ray-induced specific-locus mutation rate in newborn male mice. *Mutat. Res.* 18:63–75
115. Selby PB. 1973. X-ray-induced specific-locus mutation rates in young male mice. *Mutat. Res.* 18:77–88
116. Seperack PK, Strobel MC, Corrow DJ, Jenkins NA, Copeland NG. 1988. Somatic and germ-line reverse mutation rates of the retrovirus-induced dilute coat-color mutation of DBA mice. *Proc. Natl. Acad. Sci. USA* 85:189–92
117. Silvers WK. 1979. *The Coat Colors of Mice.* New York/Berlin/Heidelberg: Springer-Verlag. 379 pp.
118. Stallings RL, Ford AF, Nelson D, Torney DC, Hildebrand CE, Moyzis RK. 1991. Evolution and distribution of $(GT)_n$ repetitive sequences in mammalian genomes. *Genomics* 10:807–15
119. Stern C. 1968. *Genetic Mosaics and Other Essays*, pp. 27–129. Cambridge: Harvard Univ. Press. 185 pp.
120. Stoye JP, Fenner S, Greenoak GE, Moran C, Coffin JM. 1988. Role of endogenous retroviruses as mutagens: The hairless mutation of mice. *Cell* 54: 383–91
121. Sturtevant AH. 1925. The effects of unequal crossing over at the Bar locus in *Drosophila*. *Genetics* 10:117–47
122. Wallace MR, Andersen LB, Saulino AM, Gregory PE, Glover TW, Collins FS. 1991. A *de novo Alu* insertion results in neurofibromatosis type 1. *Nature* 353: 864–66
123. Wallis GA, Starman BJ, Zinn AB, Byers PH. 1990. Variable expression of osteogenesis imperfecta in a nuclear family is explained by somatic mosaicism for a lethal point mutation in the α1(1) gene (COLIAI) of type I collagen. *Am. J. Hum. Genet.* 46:1034–40
124. Whitney JB III, Lamoreux ML. 1982. Transposable elements controlling genetic instabilities in mammals. *J. Hered.* 73:12–18
125. Wijsman EM. 1991. Recurrence risk of a new dominant mutation in children of unaffected parents. *Am. J. Hum. Genet.* 48:654–61
126. Winterpacht A, Hilbert M, Schwarze U, Mundlos S, Spranger J, Zabel BU. 1993. Kniest and Stickler dysplasia phenotypes caused by collagen type II gene (COL2A1) defect. *Nat. Genet.* 3:323–26
127. Wöhrle D, Hennig I, Vogel W, Steinbach P. 1993. Mitotic stability of fragile X mutations in differentiated cells indicates early post-conceptional

trinucleotide repeat expansion. *Nat. Genet.* 4:140–42

128. Yang TP, Stout JT, Konecki DS, Patel PI, Alford RL, Caskey CT. 1988. Spontaneous reversion of novel Lesch-Nyhan mutation by HPTR gene rearrangement. *Somat. Cell Mol. Genet.* 14:293–303
129. Zheng C-J, Byers B, Moolgavkar SH. 1993. Allelic instability in mitosis: A unified model for dominant disorders. *Proc. Natl. Acad. Sci. USA* 90:10178–82
130. Zühlke C, Riess O, Bockel B, Lange H, Thies U. 1993. Mitotic stability and meiotic variability of the $(CAG)_n$ repeat in the Huntington disease gene. *Hum. Mol. Genet.* 2:2063–67