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QUALITATIVE ANALYSIS OF MOUSE SPECIFIC-LOCUS MUTATIONS: INFORMATION ON GENETIC ORGANIZATION, GENE EXPRESSION, AND THE CHROMOSOMAL NATURE OF INDUCED LESIONS

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Table of contents

1. INTRODUCTION

Mutations scored in specific-locus experiments can serve many purposes. Their primary function, of course, is to detect and measure mutagenicity of an agent.[1] This primary function is fulfilled rapidly and accurately, thanks to the well-defined phenotypes of mutations at the marked loci. However, when resources permit the setting up of stocks and the subsequent genetic analysis of the mutations, the information obtained can make major contributions to several other areas of knowledge. Such information can shed light on the nature of mutations induced by specific agents, and on the manner in which certain types of genetic lesions are expressed on the organismic level. These areas of information are needed, in combination with each other, for an assessment of risk. Thirdly, the findings shed light on the genetic organization in mammalian chromosomes. Finally, they serve to identify tools for further investigations in basic genetics and mutagenesis. This paper summarizes contributions to these areas from findings made on mutations induced in radiation experiments at Oak Ridge.

2. GENETIC MATERIAL ANALYZED

The specific-locus test (SLT) developed by W. L. Russell[2] detects forward mutations at 7 loci. Many hundreds of such mutations were scored in a variety of radiation experiments carried out over almost 3 decades, and a sizable proportion of these have been propagated as stocks. Mutations at 3 of the loci -- d (dilute), se (short ear) and c (albino) -- have been genetically analyzed. The d and se loci are closely linked (0.2 cM) on

Chromosome 9, and the c locus is located on Chromosome 7. The analysis has involved: (1) allelism tests; (2) a variety of phenotypic characterizations of the original mutant animals and their heterozygous and homozygous descendants (classification includes: whole-body vs. fractional; resembling marker allele or different allele; normal or altered viability, fertility, weight); (3) deficiency mapping with nearby markers; (4) fullscale complementation tests of the non-viable subset of mutations, using deaths at various stages of prenatal or postnatal development, body weight, and reduction or absence of various enzymes as phenotypes; (5) cytological analysis of banded chromosomes in a few of the mutants.

Our studies involved 314 independent mutations, distributed as follows: 122 d locus, 43 se locus, 37 d se,[3,4] with over 800 combinations of independent mutants studied; and 112 c locus,[5-8] with 469 combinations studied. Numerous complementation groups were delineated in each of the regions, and several new functional units identified. Two of the 112 Oak Ridge c-locus mutations and three derived from experiments at Harwell have also been analyzed by Glueckshon-Waelsch.[9]

The standard 7-locus SLT has detected not only alterations involving the marked loci themselves, but also genetic changes elsewhere on the chromosome acting through a special type of position effect.[10-12] In addition, a specific-locus test in which the hemoglobin loci, Hba and Hbb, were included as markers,[13] yielded an aberration affecting one of the standard loci, c.[14] These other products of SLTs will also be included in the subsequent discussion.

3. FINDINGS CONCERNING GENETIC ORGANIZATION

As shown by complementation maps for d and/or se mutations [4,15] and c-locus mutations (Fig. 1), the procedures used are capable of considerable resolution of mutational types and of the genetic region. The SLT is evidently capable of scoring more than a limited array of genetic alterations. Starting with 2 markers on Chromosome 9, the analysis yielded over 20 complementation groups involving 11–12 functional units, including 8–9 newly identified ones. At the c locus, the analysis yielded 13 complementation groups involving 8 functional units, including 5 newly defined ones. There is evidence (Sec. 4) that some of the complementation groups represent intragenic mutations and that others are deficiencies overlapping the marked locus or loci.

F-1

Large numbers of cistrons must lie within the spans defined by the deficiencies in each of the two regions, and the functional units identified to date provide evidence on only a small fraction of this number. Additional functional units could be defined (a) by future enlargement of the complementation grid through the inclusion of new mutants, and (b) by more extensive investigations of phenotypes in the existing grid. For example, embryological studies of c-locus mutants succeeded in subclassifying what was originally one phenotype, prenatal death, according to intrauterine stage at which death occurred. Introduction of these more detailed phenotypes into the complementation studies led to the definition of three functional units -- one for preimplantation survival and two for implantation survival.[8] Detailed analysis of physiological, morphological, and developmental characters of the mutants, singly and in all combinations, would undoubtedly produce

finer subdivisions than those achieved to date. Although it is not impossible that intracistronic complementation[16] exists in the mouse, it is likely that most functional units that can be separated by complementation represent separate cistrons.

It may be particularly useful to look for new functional units between the c locus and the two units presently mapping adjacent to it — that for neonatal survival, G6Pase, and certain other proteins on one side, and that for juvenile survival, normal size and fertility on the other. Already, over 80 distinct combinations of c-locus deficiencies extending to the left and right of c have produced what appears to be full complementation for all phenotypes studied (except albinism). If more detailed studies also fail to reveal aberrant phenotypes, one may conclude that the sites controlling the neonatal and juvenile survival functions are either immediately adjacent to the c-locus or separated from it by non-coding DNA.

One objective in developing a map of functional units within a given chromosomal segment is to obtain a sample of the mix of vital and non-vital functions in the mouse genome. Because complementation for various types of lethality is relatively easy to study, vital functions are among the first ones localized on the complementation map. Thus in the c-locus region, five new vital "loci" have been mapped (Fig. 1), and, in the d-se region, seven to eight (Russell, unpublished). The study has, however, also succeeded in demonstrating that certain loci are non-vital. Thus, combinations of certain deficiencies that overlap at c (c^{A} or c^{Ai} with c^{Bi}, c^{Bp}, or c^{C} -- see Fig. 1) can produce complementation for lethality but not for the visible effect, albinism. The c locus is thus a non-vital locus. A similar demonstration can be brought for the se locus.

There is also indirect evidence that the total absence of the Mod-2 (mitochondrial malic enzyme) locus is fully viable. While combinations of mutants (c^{E}/c^{Bi} or Bp) that lack both the MOD-2 and "juvenile-survival" functions die between days 7 and 119 of age and are of reduced size ("Pattern II"),[8] the very same syndrome is produced by combinations that lack only the "juvenile-survival" function but carry one dose of Mod-2 (namely, c^{C}/c^{Bi} or Bp, $c^{C}/c^{Di,Dp,Dj}$, or Dq, c^{C}/c^{Fp} or Fq). Absence of Mod-2 cannot thus be implicated in the Pattern-II syndrome, and a single dose of Mod-2 (as in $+/c^{E}$) is known to be compatible with survival.

The situation is less clear at the d-locus, where it has not yet been possible to achieve complementation for the opisthotonic-lethal function. Thus, combinations of overlapping deficiencies have produced the dilute-opisthotonic phenotype (over 220 such combinations), or prenatal lethality, but not viable dilutes.[4,15] It is possible that there is an "op" site within the d cistron; or, that d^{op} is the amorph and d the hypomorph with regard to a common gene product. Although one case of possible recombinational separation of d from d^{op} was observed, this case could also be explained by a spontaneous reverse mutation, $d \longrightarrow +$.[3]

In the c-locus region, it was possible to postulate an alignment of functional units by which all analyzed mutations fit a linear pattern, and there is no compelling argument against the assumption that all c-lethals are overlapping deficiencies. In the d-se region, while the majority of d^{pl}, se^{1} and dse mutants fitted a linear pattern, there were a few which did not. In tests conducted to determine whether some of these non-conforming types could be the result of two independent mutations, the results

indicated that this was not the case. However, one mutant provided evidence of conversion-like events in crossover experiments that utilized flanking markers on both sides.[4] These events have not yet been explained.

The analysis of mutations provides evidence by which the genetic map can be related to the cytological map, since some of the deficiencies are large enough to produce visible deletions in banded metaphase chromosomes. Working with a single deficiency known to contain a given locus, a crude localization can be derived.[17] A more refined cytological mapping is possible when several deficiencies involving different assortments of loci can be studied in banded chromosomes. This type of analysis has allowed us to assign c and Mod-2 to band E1 (proximal portion), sh-1 to band E1 (distal portion) or band E2, and Hbb distal to E3.[18,19] A more extensive analysis,[19] involving, in addition to deficiencies, also some other chromosome aberrations (Sec. 2), provides tentative evidence that relative distances in the genetic map may differ from those in the metaphase map.

4. FINDINGS CONCERNING GENETIC EXPRESSION

Analysis of mutations recovered in SLT experiments has provided some answers (and will provide more in the future) concerning the action of several loci and of small chromosomal regions on the organismic or cellular level. The information that can be obtained from combinations of various overlapping deficiencies extends that which can be obtained from individual homozygotes and heterozygotes for studies of the effect of either total or heterozygous absence of small chromosomal segments.

The available genetic material also permits the study of expression of non-deficiency mutations. Evidence that certain ones of the mutations are of this type exists for the hypomorphs. Thus, as discussed (Sec. 3), at c and d, overlapping deficiencies produce pigment phenotypes indistinguishable from c/c and d/d, respectively, and deficiencies overlapping at se produce an external-ear phenotype that resembles se/se. Therefore, any mutation whose phenotypic expression differs from c, d, or se in the above respects may be assumed to be due to intragenic change, rather than deficiency (Table I). Many such "intermediate alleles" have been found in radiation experiments. (T-I)

While total absence of the c or se loci is known from the study of overlapping deficiencies, to produce viable animals indistinguishable from c/c or se/se, respectively, one cannot assume that the converse is necessarily true; i.e., induced mutations whose phenotypes in the homozygous state are equivalent to those of c/c or se/se need not (but may) be deficiencies of the respective cistron. Such nulls could also be intragenic lesions (Table I). Thus, the homozygous viability of nulls at proved non-vital loci cannot, by itself, yield any information concerning the nature of the mutational lesion.

4.1. Total absence of small chromosomal segments

The homozygous effects of known deficiencies can vary over the entire possible viability spectrum, depending on location of the deficiencies. Thus, total absence of a small chromosomal segment may be (1) completely viable [e.g., overlapping Df(c)'s, Df(se)'s, or Df(Mod-2)'s], (2) lethal as late as the young adult (e.g., d^{op}/d^{op}, c^{C}/c^{C}, or combinations overlapping

at these units), (3) lethal perinatally (c^A/c^A or appropriate combinations), (4) lethal at or shortly after implantation (see Fig. 1 for homozygotes and combinations), (5) lethal before implantation (Fig. 1).

An exceptional finding that is, so far, without explanation concerns certain non-complementing combinations of c lethals. Among 221 combinations (13,209 offspring) that kill well before the midpoint of gestation, there were 9 (614 offspring) in each of which a single individual escaped from this early death and survived until birth.(8) Recombinational explanations can be ruled out for most of these cases.

For a given region, there appears to be a crude relation between length of the deficiency and time of death of the homozygote; for example, all deficiencies extensive enough to be readily detectable in banded metaphase chromosomes (c^{Fp1}, c^{Fq1}, c^{Dq1}, and $c^{Dp[or\ Dq]25H}$) kill before implantation. However, specific content of a deficiency is presumably a more important determinant than length. It is conceivable, e.g., that, on the tp side of c (see Fig. 1), the nearest functional unit for preimplantation survival could be at a considerable distance from the last functional unit mapped; if so, one might recover deficiencies that are longer than the preimplantation lethal c^{Bp} group but kill later, namely at or soon after implantation.

4.2. Heterozygous deficiencies

The analysis of SLT mutations has shown that surprisingly long deficiencies are recoverable in heterozygous condition (Table II). In the c region, two of the deficiencies are at least 6 cM long (but neither of these could be greater than 11 cM). In the d-se region, the longest deficiencies found were at least 2.2 cM, but at most 11 cM, in length.

T-II

While exact determination of vital effects of heterozygous deficiencies (survival, weight, reproductive capacity, etc.) must await analysis in coisogenic stocks, it is already clear that marked viability depression occurs in certain cases. As was true for homozygous effects, content is probably a more important determinant of heterozygous viability than is length (Table II). Thus, many c locus deficiencies that are longer than 2 cM produce no readily measurable effects in heterozygotes, while marked viability depression was found for an se-lethal that is less than 2.2 cM long.

There appears to be some correlation between the presence of clear heterozygous effect and early time of death of the homozygote. Thus c-locus-mutant stocks segregating for heterozygous and wild-type offspring were studied with respect to whether there was a reduction in the expected numbers of the former. In 13 stocks of preimplantation lethals and 21 stocks of lethals that kill at later stages, heterozygote frequencies were significantly reduced in 31% and 5% of the stocks, respectively ($P = 0.11$ for difference between the two groups of stocks), and obviously (but not necessarily significantly) reduced in 54% and 10% of the stocks, respectively ($P = 0.01$).

The analysis of specific-locus mutations has provided some clues about the chromosomal nature and heterozygous effects of the genetic alterations that may be recovered in recessive-lethal tests in the mouse.[20,21] Such recessive lethals could be deficiencies ranging from very small to quite long (at least 6 cM), and some of them may be intragenic mutations. The heterozygous effects of recessive lethals could range from not obviously

deleterious to markedly so, and the latter type is expected to be more prevalent among those lethals that kill before implantation.

4.3. Associated phenotypes; pleiotropy

The large numbers of characterized deficiencies that are available, and the extensive complementation grids that can be constructed with them, permit tentative conclusions as to whether certain phenotypes that are found associated in some mutants are multiple end points of a single basic lesion, or the result of the loss of several separate, though neighboring, cistrons. An example is provided by the association of the lack of glucose-6-phosphatase (G6Pase) activity and perinatal death that occurs in homozygous $\underline{c}^{A}$-group $\underline{c}^{E}$-group mutants. We were able to produce 97 neonatally lethal combinations of independent mutations (involving 9 complementation groups), and deHamer[22] tested 70 of these for G6Pase activity; every one was severely deficient. Of 117 combinations that did not die neonatally, 87 were tested for G6Pase, and in all 87 of these the activity was in the range of that found in normal littermates. Thus, the two phenotypes could not be separated in extensive tests and probably result from the same basic lesion.

A number of additional phenotypes have also been found in certain homozygous $\underline{c}^{A}$- and $\underline{c}^{E}$-group mutants, namely, abnormality of two other liver-specific enzymes, of serum protein, and of the structure of subcellular membrane organelles.[9] So far, an association of these abnormalities with G6Pase deficiency has been demonstrated in 3 combinations of mutants, while 3 other combinations, which had normal G6Pase levels, were normal in the other respects also.[23] While these numbers are not as extensive as those for the G6Pase-neonatal-death

comparison, they provide some evidence of the same sort, suggesting that all of the multiple phenotypes are part of a single basic lesion at what is assumed to be a regulatory locus.[9] By contrast, as discussed in Sec. 3, lesions at the Mod-2 locus presumably do not have associated viability phenotypes.

4.4. Copy number

The ability to study the effects of certain regions in double, single, and sometimes zero dose (in certain combinations of overlapping deficiencies) allows the simple determination of whether product is related to copy number. This is the case for Mod-2,[24] the structural locus for mitochondrial malic enzyme; on the other hand, G6Pase activity is the same in heterozygous deficiencies as in normal genomes, and Gluecksohn-Waelsch[9] has taken this as evidence that a regulatory gene is involved (see also Sec. 4.3). No specific gene product is known for the other functional units that control survival at various ages; however, it should be noted that whenever complementation for lethal effects was found, it was full rather than partial complementation, indicating that a single copy of the pertinent region was sufficient.

4.5. Gene expression at the cellular level

Some attempts have been made to study the expression of genes located at or near the marked loci on levels besides the organismic one. One major tool for such investigations is provided by X-autosome translocations affecting Chromosomes 7, on which c and p are located, and Chromosome 4, on which lies the b locus. In such translocations, major portions of the pertinent autosomal region are inactivated in roughly half the cells of the

body.[10-12] We have studied the effects of p, c, and b lethals (presumed deficiencies) carried in the non-translocated autosome opposite reciprocal X-autosome translocations that carry the pertinent wild-type allele.[25,26,15] As illustrated in Fig. 2, females with such genomes are functional mosaics of the type +/Df(m)///0/Df(m) [where Df(m) represents a deficiency for the marked locus and probably adjacent regions, and $+^m$ represents the wild-type allele at the marked locus and intact adjacent regions]. With respect to dosage of $+^m$, such females are 1///0 mosaics, and -- if a zero dose of $+^m$ is viable on the cellular level -- there should be phenotypic mosaicism for the marker (namely, wild type where the cellular genotype is $+^m/0$; and the color characteristic of the homozygous null allele where the cellular genotype is 0/0). F-2

The results of these experiments indicate whether the deficiencies, in addition to being organismic lethals (a fact already known from the Df(m)/Df(m) genotypes), also act as cell lethals; if so, no coat-color mosaicism would be obtained. The findings were different for different deficiencies tested (Table III): 12 independent Df(b)'s and a Df(p) were not cell lethal but depressed overall viability in the T/Df combinations,[25] indicating that dosage of some gene product was important on the organismic level; deficiencies of the c^A and c^E groups did not markedly affect cell viability or overall viability; and a c^{Dj}-group deficiency prevented survival when in combination with T(X;7).[15] Gluecksohn-Waelsch[9] has recently reported on a similar study in which 5 c lethals were combined with the TlCt X insertion. Her results for a c^A-group mutant and for two c^E's parallel ours in terms of showing full, or near-full, overall viability. Survival was zero for the combination with c^{25H} [probably a c^{Dp}- or T-III

$\underline{c}^{Dq}$-group mutant[8]], thus paralleling our findings with $\underline{c}^{Dj}$. It was clearly depressed with $\underline{c}^{6H}$ (a $\underline{c}^{Bi}$-group mutant), similar to the condition we had reported for Df($\underline{b}$) and D($\underline{p}$) mutants.[25]

The problem of possible cell-lethal (or semi-lethal) action of deficiencies arises, in a specialized setting, in the case of the gametes. It was found some time ago[3] that several of the $\underline{d}$ $\underline{se}$ deficiencies were transmitted with less than normal frequency. While such reduced transmission ratios could be the result of selection against heterozygous embryos, the evidence for selection in gametes comes from the finding that the depressed ratios also occurred in the progeny of heterozygous males that produced normal littersizes (see Fig. 6 in Ref. 27). If more stringent evidence confirms this indication of gametic selection, it may be possible to identify gene products expressed in normal gametes which are missing in deficiency-bearing gametes.

5. FINDINGS CONCERNING THE MUTATION PROCESS

5.1. Radiation-induced mutations

The characterization of SLT mutations has allowed certain conclusions to be drawn about what types of mutations are (or are not) induced by radiation, and how the biological and physical variables of the treatment affect the nature of the mutations produced. It could be demonstrated (Sec. 4) that "intermediate alleles," at least at the $\underline{c}$, $\underline{se}$ and $\underline{d}$ loci, must be intragenic mutations, and that radiation is thus capable of producing such mutations as well as deficiencies. The majority of radiation-induced mutations at the $\underline{c}$ and $\underline{se}$ loci are to the null allele and are homozygous viable ($\underline{c}^{av}$ or $\underline{se}^{v}$). Since these two loci have been

shown to be "non-vital" loci, such mutations could be deficiencies no larger (or not much larger) than the locus itself; they might also be intragenic mutations (Table I). Mutations that produce a null phenotype for the marker locus and affect other functions as well (e.g., viability, though the marked locus is "non-vital") are multi-locus deficiencies.

The type of mutation induced has been shown to be correlated with the germ-cell type irradiated and with the ion density of the radiation applied, but not with dose rate.[4,8] Irradiation of spermatogonial stem cells produces more restricted lesions (i.e., more hypomorphs, fewer multi-locus deficiencies) than does irradiation of postspermatogonial stages or oocytes. Within the cell-stage groups, low-LET irradiation produces more restricted lesions than does neutron irradiation. These relations are illustrated in Table IV for the d-se and c regions. (T-IV)

The demonstration of the relatively restricted nature of mutations induced even at high dose rates by low-LET irradiation in spermatogonial stem cells made possible the conclusion that single-track events are responsible for most such mutations. The decrease in mutation rate by dose protraction, which had earlier been demonstrated for spermatogonia,[28] thus could not be explained in terms of reducing the chance for interaction of two separate hits. The analysis of mutations has therefore strengthened the original hypothesis that the dose-rate effect is accounted for on the basis of single-track mutational lesions, with a swamping of the repair process at high dose rates.

Spermatogonial exposure to ethylnitrosourea produces an even higher frequency of hypomorphs and lower frequency of multilocus deficiencies (lethals) than does low-LET irradiation of spermatogonia.[29] Thus, the

overall relative frequency of nulls to altered-activity mutants depends on the nature of the mutagen and the type of cell exposed. For intragenic mutations alone, keeping mutagen and exposed cell type constant, the relative frequency of nulls and hypomorphs undoubtedly varies with the locus.

5.2. Spontaneous mutations

The detailed study of SLT mutants has shown that the pathways by which spontaneous mutations arise may differ from those involved in the induction of mutations. The frequency of fractional (mosaic) mutants at all 7 loci is roughly similar in offspring of irradiated and control mice,[25] and the same is true when the study is restricted to the c locus.[6] Radiation thus does not induce fractionals, and fractional mutants in irradiated as well as control groups are presumably of spontaneous origin. At the c locus, the large majority of spontaneous mutations were fractionals, and, at d, almost one-half were. (Fractionals would probably be nondetectable at se, and more poorly detectable at d than at c). Analysis of segregation ratios derived from 16 c-locus fractionals led to the conclusion that the mutations had occurred in one strand of the gamete DNA, or in a daughter chromosome derived from pronuclear DNA synthesis of the zygote, or in one of the first two blastomeres prior to replication.

Another mechanism implicated in the production of certain spontaneous mutations is "double nondisjunction," whereby the offspring receives 2 copies of the marked chromosome and no copies of the wild-type chromosome. Several spontaneous d se mutants[3] as well as an $\frac{Hbb^d}{}$ $\frac{c^+}{}$ mutant[13] are apparently of this type. The event is detectable only where two markers are present on the chromosome involved. When only one marker is present,

the result is indistinguishable from a repeat mutation to the marker allele. Notwithstanding its name, "double *nondisjunction*" could result from a single nondisjunctional or recombinational event in the first (or an early) cleavage. Although such an event produces two genetically distinct blastomere populations, the embryo proper would often be nonmosaic, because it arises from only a very small subset of the cleavage products. There is no evidence that either mosaic or "double nondisjunction" mutants are induced by radiation or other mutagenic exposure of the germcells. Indeed, one would not expect them to be if they arise in the zygote or shortly thereafter, as postulated. The population of spontaneous mutants might therefore have an admixture of types not present in the population of induced mutants. This possibility raises doubts concerning the accuracy of the "doubling-dose" approach in the calculation of genetic risk.

6. FINDINGS THAT PROVIDE TOOLS FOR BASIC INVESTIGATIONS

The analysis of *SLT* mutations has yielded, in addition to presumed intragenic alterations, a considerable number of chromosomal aberrations. These include series of deficiencies that overlap to various degrees and extend to varying lengths in both directions from the marker[4,8]; X-autosome translocations that (in about half the cells of the body) inactivate regions including some of the marked loci[11,12]; and a tandem duplication which duplicates the c-Hbb segment as well as a region on either side.[14] In addition, it is possible that the se-locus mutations that do not fit the linear complementation map (Sec. 2) may represent small rearrangements, perhaps inversions (Russell, 1971).

Using the array of characterized aberrations, it is possible to construct extensive series of gene dosages ranging (organismically) from 0 to 3 copies in steps of 0.5 (Fig. 4 in Ref. 27). Such dosage series can be used to investigate not only the marked loci, but any cistron included in some of the deficiencies, e.g. Mod-2, or its specific regulator, Mdr-1.[30] Such dosage series can greatly expand the comparison of 2, 1, and 0 copies, discussed in Sec. 4.4., and may serve to identify additional structural or regulatory loci. In addition to comparisons of gene dosages, the material also permits comparisons of cis and trans effects by utilizing the Dp/Df combination for the former configuration.

The deficiencies can be useful for the mapping of genes whose location is only approximately known, (e.g. from cell-hybridization studies), particularly those for which no variants have been identified. For example, it is known that the mouse Ldh-1 (lactate dehydrogenase α chain) gene is on Chromosome 7. LDH dosage studies in Df(p) and Df(c) mutants might lead to definitive localization of Ldh-1. New genes could be identified (and localized) by comparing normal and heterozygously deficient animals with respect to certain enzyme activities. Deficiency mapping can also establish order of genes in cases where only distances are known. Thus, sv was localized on the opposite side of se from d by this method.[31]

The characterized mutations can provide material for attempts at genetic "rescue." For example, it might be possible to determine whether the small body size associated with the Chromosome-7 tandem duplication can be eliminated by combining this duplication with certain deficiencies spanning part of the same region. If such attempts were successful, this would lead to an approximate localization of major body-size genes.

Genetic rescue has been attempted for the male-sterilizing effects of T(X;7)s -- however without success.(32)

The use that can be made of T(X;A)/Df combinations in determining whether certain genetic states are cell lethal was discussed in Sec. 4.5.

The various chromosome aberrations identified among SLT mutations may, finally, provide favorable material for studies designed to identify and isolate DNA sequences from genetically defined regions of the mouse genome. The methodology for such studies has been proposed (Russell and Bernstine, 1982), and experiments are in progress to identify DNA restriction fragments which are missing in a large c-locus deficiency.(33)

7. SUMMARY

Analysis of mouse specific-locus (SL) mutations at three loci has identified over 33 distinct complementation groups -- most of which are probably overlapping deficiencies -- and 13 to 14 new functional units. Perhaps due to ease of ascertainment, the complementation maps that have been generated for the d-se and c regions include numerous vital functions; however, some of the genes in these regions are non-vital, i.e., the mouse can tolerate their total absence as produced by overlapping deficiencies. At such loci, hypomorphic mutants (as distinguished from nulls) must represent intragenic alterations, and some viable nulls could conceivably be intragenic lesions also.

Analysis of SL mutations has provided information on genetic expression. Homozygous deficiencies can be completely viable or can kill at any one of a range of developmental stages. Heterozygous deficiencies of up to 6cM or more in genetic length have been recovered and propagated.

The time of death of homozygous deficiencies and the degree of inviability of heterozygous deficiencies are probably related more to specific content of the missing segment than to its length. — Multiple phenotypes that are found associated in some of the mutants may result from a lesion affecting a single gene, or from the loss of several neighboring cistrons; and the distinction between these alternatives has been aided by complementation analysis. — Combinations of deficiencies with X-autosome translocations that inactivate the homologous region in a mosaic fashion have shown that organismic lethals are not necessarily cell lethal.

The spectrum of mutations induced (e.g., the ratio of nulls to altered-activity mutants) depends on the nature of the mutagen and the type of germ cell exposed. Radiation of spermatogonia produces intragenic as well as null mutations. Spontaneous mutations (several of which may arise in the zygote or in early cleavage) have an admixture of types not present in populations of mutations induced in germ cells, and this raises doubts concerning the accuracy of "doubling-dose" calculations in genetic risk estimation.

The analysis of SL mutations has yielded genetic tools for the construction of detailed gene-dosage series, cis-trans comparisons, the mapping of known genes and identification of new genes, genetic rescue of various types, and the identification and isolation of DNA sequences.

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Table I. Relation between phenotype of mutant at specific locus[a] and probable chromosomal nature of mutation

Expression of mutation at marked locus	Change in other phenotype(s)	Probable chromosomal nature of mutation
Hypomorph	No	Intragenic lesion
Null	No	Intragenic lesion; or deficiency of marked locus only[b]
Null	Yes	Multilocus deficiency

[a]Established for c and se loci

[b]or including adjacent non-coding DNA

Table II. Selected findings concerning heterozygous deficiencies

A. Maximum lengths recovered as viable heterozygotes

Region	Type	Length (cM)
c	Df (c Mod-2 sh-1)	6 - 11
d-se	Df (d se sv)	2.2 - 11

B. Examples showing that content, more than length, affects viability

Deficiency	Length (cM)	Viability of +/Df
Df(c Mod-2)	2 - 9	Normal[a]
Df(se)[b]	0 - 2.2	Markedly reduced

[a]Not distinguishable from +/+ on mixed background.(5) Not yet analyzed in co-isogenic stocks.

[b]Mutant se^{207K}. Ref. 34.

Table III. Phenotypes of females carrying X-autosome translocations and deficiencies involving specific loci

Translocation-deficiency combinations[a,b]	Phenotype	
	Viability	Coat-color mosaicism
$\frac{T(X;7)}{c^A \text{ or } c^E}$ (15) $\quad \frac{TlCt}{c^A \text{ or } c^E}$ (9)[c]	near-normal	yes
$\frac{T(X;4)}{Df(b)}$ (25)[d], $\frac{T(X;7)}{Df(p)}$ (25), $\frac{TlCt}{c^{Bi}}$ (9)	reduced	yes
$\frac{T(X;7)}{c^{Dj}}$ (15) $\quad \frac{TlCt}{c^{Dp \text{ (or } Dq)}}$ (9)	lethal	--

[a] See Fig. 2 for illustration of combination involving reciprocal translocation and deficiency. For extent of deficiencies designated by the c-locus complementation-group symbols, see Fig. 1. Time of death of homozygous deficiencies as follows: c^A/c^A, or c^E/c^E, perinatal; c^{Bi}/c^{Bi}, c^{Dj}/c^{Dj}, or Df(p)/Df(p), at or shortly after implantation; c^{Dp}/c^{Dp} or c^{Dq}/c^{Dq}, before implantation; the 12 Df(b)/Df(b)'s, not yet determined.

[b] Superscript numbers refer to publication list.

[c] TlCt, is an insertion of a large segment of Chromosome 7 into the X.

[d] 12 independent Df(b)'s were tested.

Table IV. Relation between circumstances of exposure and type of mutation[a]

Germ-cell stage	Type of radiation	d-se region				c-region			
		No.	Hypomorph %	Null; no other phenotype %[b]	Null; also other pheno. %	No.	Hypomorph %	Null; no other phenotype %	Null; also other pheno. %
Spontaneous	-	28[c]	7.1	89.3	3.6	17[c]	29.4	64.7	5.9
Spermatogonia	Low LET	81	7.4	75.3	17.3	51	17.6	49.0	33.4
Spermatogonia	Neutrons	39	5.1	61.5	33.3	15	0	60.0	40.0
Postgonial stages	Various	25	0	56.0	44.0	8	0	37.5	62.5
Oocytes	Various	29	3.4	24.1	72.4	16	0	37.5	62.5
Postgonial + oocytes	Low LET	45	2.2	40.0	57.8	12	0	50.0	50.0
Postgonial + oocytes	Neutrons	9	0	33.3	66.7	12	0	25.0	75.0

[a]See Table I for probable chromosomal nature of mutations.

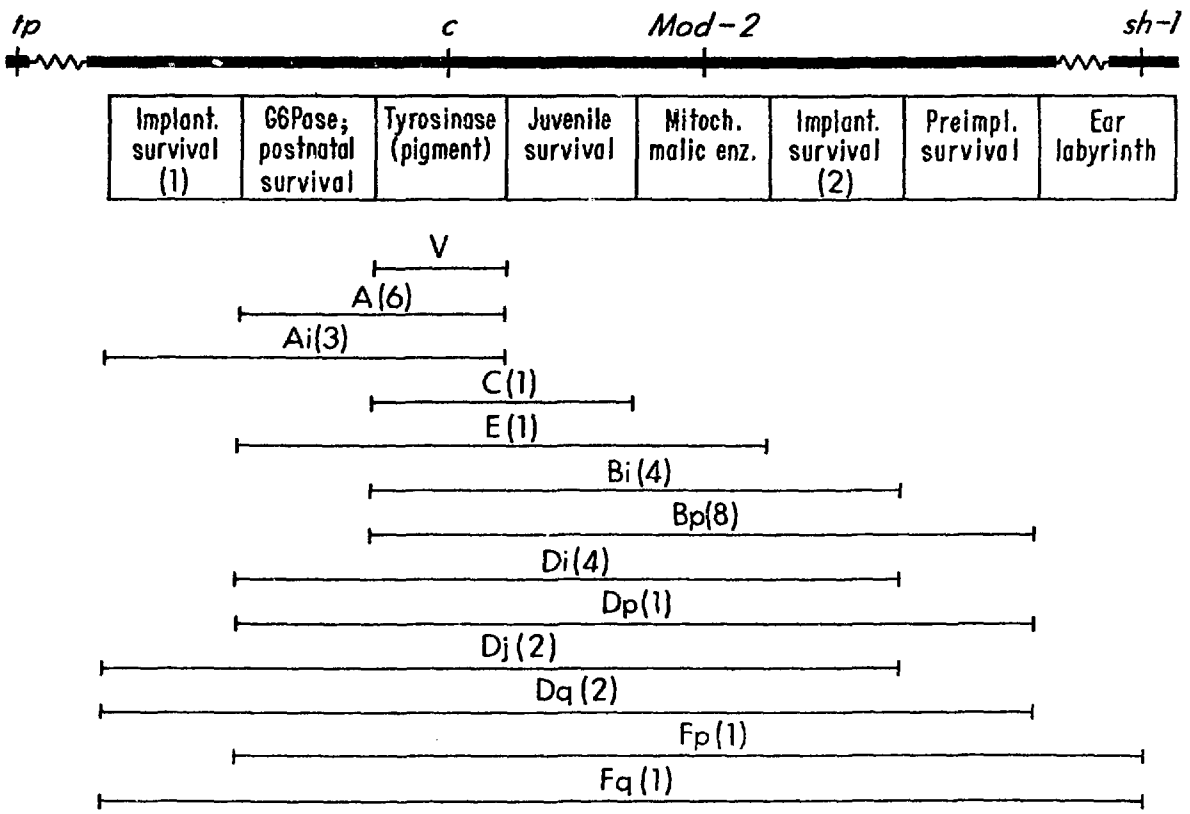
[b]Includes d^{op}

[c]Includes fractional (mosaic) mutants from experimental groups (see text).

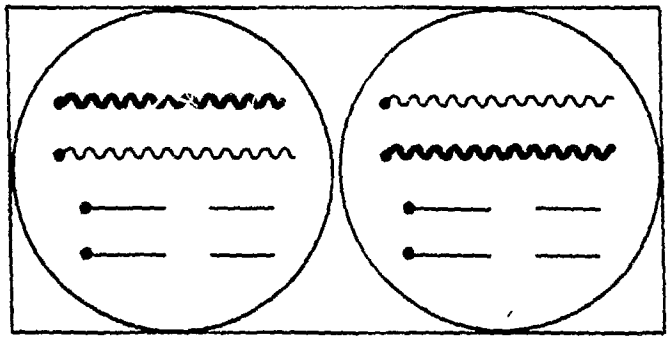
FIGURE LEGENDS

FIGURE 1. -- Complementation map of c-locus mutants developed by Russell et al.[8] With postulated functional units (shown in boxes below the genetic map), all mutants fit a linear pattern. The number shown in parentheses following each complementation-group designation indicates the number of independent Oak Ridge mutations in the group. In addition, Harwell mutants c^{3H}, c^{6H}, and c^{25H}, can probably be added to groups E, Bi, and Dp (or Dq), respectively. "V" indicates viable albino mutants of which there were 52 among 90 non-mosaic c-locus mutations found in the progeny of irradiated mice.

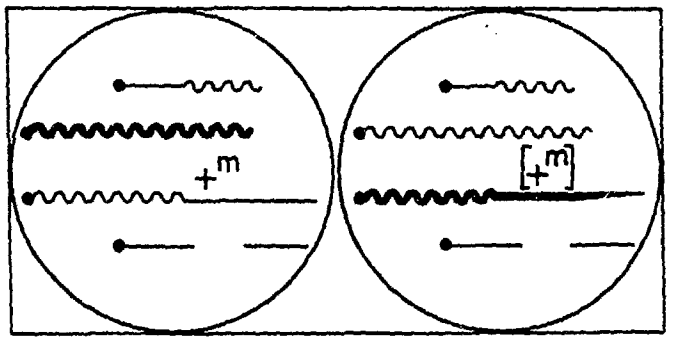
FIGURE 2. -- Use of X-autosome translocations as tools to explore whether organismically-lethal deficiencies act also as cell lethals. Straight lines, autosomes or autosomal segments; wavy lines, X-chromosomes or X-chromosomal segments; heavy wavy or heavy straight lines, inactivation due to X allocycly; gap in autosome, deficiency of marked locus m and, possibly, adjacent loci; $[+^{m}]$, inactive gene. Alternative cellular conditions are shown for a female deficiency homozygote, Df(m)/Df(m) (left rectangle), and for a female heterozygous for an X-autosome translocation carrying $+^{m}$, and for Df(m) in the intact autosome (right rectangle). See Table III for various observed outcomes.


tp
c
Mod-2
sh-1
Implant. survival (1)
G6Pase; postnatal survival
Tyrosinase (pigment)
Juvenile survival
Mitoch. malic enz.
Implant. survival (2)
Preimpl. survival
Ear labyrinth
V
A(6)
Ai(3)
C(1)
E(1)
Bi(4)
Bp(8)
Di(4)
Dp(1)
Dj(2)
Dq(2)
Fp(1)
Fq(1)

Deficiency homozygote



Translocation – deficiency combination



Dose of $+^m$	0	1 /	0
Cellular phenotype:	–	$+^m$ /	m (if cell viable)
Organismic viability:	No	?	