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IMPROVED MUTAGEN TESTING SYSTEMS IN MICE

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Final Report to Department of Energy

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Our work was initiated in a direct response to the need for a mutagen test system in mice that made possible the ascertainment of recessive lethals and detrimentals in mammals. This need was made clear in a symposium we organized held at the Jackson Laboratory in (Proceedings of the symposium contained in the Appendix). It was Dr. Charles Edington of the Department of Energy (then the Atomic Energy Commission) who, with his experience in Drosophila genetics and mutagenesis called for the production of chromosomal inversions in experimental mammals, and their development to produce a system in mammals to ascertain (and maintain for research) recessive deleterious and lethal mutations. Four laboratories were then supported to do this work, each with different approaches.

Our laboratory was the first to induce and ascertain a mammalian chromosomal inversion; we did this by searching for a high frequency of first meiotic anaphase bridges in testes of males whose fathers received post-spermatogonial radiation or mutagenesis from chromosomal breaking chemical mutagens. One testis was examined in each mouse, and those showing a high frequency were then mated to determine if the high frequency were passed on as a dominant and whether linkage analysis suggested the presence of an inversion. A very high incidence (exceeding 20% bridges in first meiotic anaphase bridges) was found in about 1 in 150 males examined and this frequency was generally found to be passed on to the offspring as predicted. Later cytological banding techniques were developed elsewhere and we used them to show visually the inverted orders of the inverted chromosomal segments. Since that time we have induced inversions covering most of the mouse genome. In the appendix is a linkage map of the inversions showing their position on their respective chromosomes based on linkage with specific markers. Also since our initial work, other laboratories have found and verified inversions, particularly the very important large X chromosomal inversion In(X)1H, based on similar biological peoperties to those inversions in our laboratory. Now, today, it is possible to verify presence of most large inversions by the karyotype analysis and direct observation of an inverted chromosomal segment.

Table 1 is a summary of the inversions produced in our laboratory. Many interesting properties of the inversions themselves have come from this study including (1) there is no evidence of position effects, and (2) about 1/3 are lethal, otherwise there is no evidence of any biological effect associated with the property of inversion alone .

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TABLE 1. INVERSIONS INDUCED IN THE MOUSE 1 Jan. 1993 T.H.Roderick

Animal number	Inversion symbol	Ana. Br. frequency		Induced by *	Induced on	Comments
471		.379+/-	.033	800 R(X)	Ra, Sd	functionally sterile
587		.176	.034	"	DBA/2J	died before mating
618	In(1)1Rk	.340	.008	900 R(X)	"	[Homo][F]
639		.151	.026	"	"	fertile, discarded
816	In(5)2Rk	.192	.010	850 R(X)	"	[Homo][F]
2414		.340	.048	900 R(X)	"	small, died
2425		.158	.033	"	"	discarded
2457	In3Rk	.143	.016	"	"	discarded
2576		.164	.050	"	"	discarded
2624		.175	.050	"	"	discarded
2913	In4Rk	.188	.013	"	"	lost
2916	In(2)5Rk	.345	.014	"	"	[Het][F]
3563	In(10)6Rk	.262	.018	"	"	[Homo][F]
4587	In7Rk	.150	.024	800 R(X)	"	discarded
4609	In8Rk	.179	.019	"	"	lost
5204	In(5)9Rk	.737	.020	TEM .2	C3D2F1	[Homo][F]
6567	In(1)10Rk	.741	.033	EMS	"	functionally sterile
6845	In(3)11Rk	.294	.055	TEM .2	C57BL/6J	[Homo][F]
6857	In(1)12Rk	.228	.047	"	"	[Homo][F]
7877	In(7)13Rk	.447	.040	"	C3D2F1	[Homo][F]
8677	In(8)14Rk	.347	.043	"	DBA/2J	[Homo][F]
10434	In(3)15Rk	.689	.038	"	B6D2F1	functionally sterile
11318	In16Rk	.458	.059	TEM .3	DBA/2J	T(1;In8)1Rk, sterile
11323	In(10)17Rk	.648	.040	"	"	pg mutation within inv.
11449	In(15)18Rk	.366	.024	"	"	[Homo][F]
11675	In(2)19Rk	.609	.035	"	"	[Homo][F]
low fat diet necessary to maintain this stock						
11684	In(11)20Rk	.489	.052	"	"	litter size reduced
in inversion heterozygotes; homozygotes have normal fertility; [F]						
11865	In(15)21Rk	.469	.032	TEM .4	"	homo. lethal; [F]
smaller testis and sperm deficiency, but normal litter size.						
11951	In(14)22Rk	.663	.036	"	"	homozygous; [F]
12164	In(1)23Rk	.364	.040	"	"	possibly In(1)1Rk;
homozygous viable and fertile						
12174	In(1)24Rk	.728	.046	"	"	induced with T(1:13)2Rk
homozygous with and without the translocation; [F]						
12452	In(12)25Rk	.459	.048	"	"	homo. lethal; [F]
12953	In(9)26Rk	.237	.028	"	"	cytologically ident.;
homozygous; [F]						
16567	In27Rk	.500	.067	TEM .3	POS (M)	died before mating
16647	In(4)28Rk	.559	.085	"	POS (AxD)	homo. lethal; [F]
7780IJ	In(11.13LS)29Rk	***		"	POS (M)	cytologically ident.
21716	In(5)30Rk	.270	.047	TEM .4	POS (F)	[Homo][F]
new retinal degeneration						
21923	In(13)31Rk	.700	.102	TEM .3	"	[Homo][F]
25022	In(4)32Rk	.211	.029	TEM .5	POS (P)	homo. lethal; [F]
inversion induced in Rb but now maintained only without Rb.						
25086	In(5)33Rk	.594	.059	Spontaneous	POS (M)	homozygous; [F]
inversion induced within Robertsonian						
25094	In(8)34Rk	.374	.045	TEM .5	POS (P)	homo. lethal; [F]
25382	In(15)35Rk	.107	.019	"	DBA/2J	homo. lethal; [F]

(continued)

29646	In(14)36Rk	.426	.042	1000R(Cs)		"	[Homo][F]
29726	In(19)37Rk	.591	.105	"		"	[Homo][F]
31189	In(1)38Rk	.531	.088	"		"	[Homo][F]
	full cataract beginning at 2 months of age						
32049	In(9)39Rk	.840	.052	"		"	[Homo][F]
32708	In(2)40Rk	.629	.082	"	POS (S)		[Homo][F]
33378	In41Rk	.320	.066	"		"	functionally sterile
34792	In(10)42Rk	.462	.052	"	DBA/2J		[Homo][F]
35108	In(13)43Rk	.236	.073	"	POS (S)		under study;
	possibly an associated reciprocal translocation with Chr. 17						
35289	In44Rk	.270	.073	"		"	functionally sterile
36073	In(2)45Rk	.730	.056	"		"	[Homo]
37281	In(1)46Rk	.440	.049	"	DBA/2J		[Homo][F]
37465	In(6)47Rk	.378	.054	"		"	[Homo][F]
37720	In(6)48Rk	.577	.069	"		"	homo. lethal; [F]
37908	In(1)49Rk	.583	.082	"		"	homo. lethal
38427	In(13)50Rk	.825	.023	"		"	cytol.det;homo. lethal
38991	In(16)51Rk	.143	.037	"		"	cytol.det;lit.size low;
	probably an inverted insertion, (T16;7) poor breeders						
39009	In52Rk	.464	.094	"		"	functionally sterile;
	induced along with 3 new translocations						
39014	In53Rk	.487	.080	"		"	functionally sterile;
39166	In(14)54Rk	.523	.075	"		"	cytol.det;homo.lethal;
	perhaps an associated deletion; poor breeders; simul. induced T(5;15)						
39257	In(3)55Rk	.702	.067	"		"	[Homo][F];
	cystic enlarged kidneys, low hematocrit,some preweaning mortality						
40492	In(4)56Rk	.833	.062	"		"	homo. lethal;
	dominant retinal degeneration						
41240	In(10)57Rk	.202	.040	"		"	fertile
41652	In(2)58Rk	.441	.076	"		"	fertile
****	In(4)1By	.236	.042	spontaneous on BALB/cBy, homozygous;			
	carries BW13 and b which the inversion probably overlaps						
****	In(4)1Dn	.027	.027	1000R(Cs)	DBA/2J		cytol.det.in female;
	fertile, no loops in s.c., 1/37 bridges; homozygous; [F]						
****	In(11)2Dn	.087	.042	"		"	cytol.det.in female;
	induced with T(9;19)37Dn						discarded, small inv.
****	In(2)3Dn	.343	.058	"		"	cytol.det.in female;
	perhaps homozygous lethal; poor breeder						
****	In(6)4Dn	.312	.082				cytol.det.in female;
	induced with T(2;11)4Dn						lost
****	In(1)5Dn (no bridged sons)			"		"	cytol.det.in female
****	In(2)6Dn (no bridged sons)			"		"	cytol.det.in female
****	In(17)7Dn						

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* 'X' indicates induced by X-rays delivered by a GE Maxitron 250 KVP at 20 MA, 1mm Cu, 1mm Al filtration.
'Cs' indicates induced by a Shepherd Mark I Cesium 137 irradiator.
The numbers by TEM and EMS indicate dosage in mg/kg body weight.

** Symbols represent strains, stocks, or hybrids. POS are stocks carrying Robertsonian chromosomes.

*** Identified first in a female by blood culture and G-banding. A pericentric inversion will not produce anaphase bridges in the first meiotic division.

**** Given to us by collaborators M. T. Davisson and D. W. Bailey;

[Homo] means available in homozygous condition.

[Het} means available only as heterozygotes. Homozygotes are probably lethal.

[F] means the stock has been successfully frozen down in embryo state.

Inversion symbols:

'In' refers to inversion.
Number in parenthesis is the chromosome in which the inversion occurs.
Number following parenthesis or alone is the number of the inversion found in our laboratory.
'Rk' = Roderick; 'Dn' = Davisson; 'By' = Bailey

As of the writing of this report nearly all these inversions are extant as caged animals on the shelf. Soon, however, most will be available only as frozen embryos which if desired be thawed for use and breeding. Table 2 shows the availability in the frozen state.

Table 2

Status of Available Inversions and Deletions From Roderick's Lab
1 Feb. 1993

Animal number	Inversion symbol	On shelf as	Whether in freezer
618	In(1)1Rk	Homozygotes	[F]
816	In(5)2Rk	Homozygotes	[F]
2916	In(2)5Rk	Heterozygotes	[F]
3563	In(10)6Rk	Homozygotes	[F]
5204	In(5)9Rk	Homozygotes	[F]
6845	In(3)11Rk	Homozygotes	[F]
6857	In(1)12Rk	Homozygotes	[F]
7877	In(7)13Rk	Homozygotes	[F]
8677	In(8)14Rk	Homozygotes	[F]
11323	In(10)17Rk	Heterozygotes	[F]
11449	In(15)18Rk	Homozygotes	[F]
11675	In(2)19Rk	Homozygotes	[F]
11684	In(11)20Rk	Heterozygotes	[F]
11865	In(15)21Rk	Heterozygotes	[F]
11951	In(14)22Rk	Homozygotes	[F]
12164	In(1)23Rk	extinct on shelf	[F as heterozygotes, only]
12174	In(1)24Rk	Homozygotes	[F]
	In(1)24Rk with intersecting translocation T(1:13)2Rk		
		Homozygotes	[110]
12452	In(12)25Rk	Heterozygotes	{F]
12953	In(9)26Rk	Homozygotes	[F]
16647	In(4)28Rk	Heterozygotes	{F]
21716	In(5)30Rk	Homozygotes	[F]
21923	In(13)31Rk	Homozygotes	[F]
25022	In(4)32Rk	extinct on shelf	[F as heterozygotes, only]
25086	In(5)33Rk	Homozygotes	[F]
25094	In(8)34Rk	extinct on shelf	[F as heterozygotes, only]
25382	In(15)35Rk	Heterozyotes	[F]
29646	In(14)36Rk	Homozygotes	[F]
29726	In(19)37Rk	Homozygotes	[F]
31189	In(1)38Rk	Homozygotes	[F]
32049	In(9)39Rk	Homozygotes	[F]
32708	In(2)40Rk	Homozygotes	[F]
34792	In(10)42Rk	Homozygotes	[F]
35108	In(13)43Rk	Heterozygotes	[300]
36073	In(2)45Rk	extinct	---
37281	In(1)46Rk	Homozygotes	[F]
37465	In(6)47Rk	Homozygotes	[F]
37720	In(6)48Rk	Heterozygotes	[F]
37908	In(1)49Rk	Heterozygotes	[F]
38427	In(13)50Rk	Heterozygotes	[F]
8991	In(16)51Rk	Heterozygotes	[F]
39166	In(14)54Rk	Heterozygotes	[F]
39257	In(3)55Rk	Homozygotes	[F]
40492	In(4)56Rk	Heterozygotes	[F]
41240	In(10)57Rk	Heterozygotes	[928]

(continued)

41652	In(2)58Rk	Heterozygotes	[703]
****	In(4)1By	Homozygous	[F]
****	In(4)1Dn	Homozygous	[F]
****	In(2)3Dn	Heterozygous	[803]
****	In(1)5Dn	Heterozygous	discarded
****	In(2)6Dn	Heterozygous	discarded
****	In(17)7Dn	Heterozygous	discarded

Lethals maintained in Roderick's Lab

l(7)-1	Heterozygous	[F]
l(1)-1	Heterozygous	---
l(1)-2	Heterozygous	---
l(1)-3	Heterozygous	---
l(1)-4	Heterozygous	---
l(1)-5	Heterozygous	---
l(1)-6	Heterozygous	---
l(1)-7	Heterozygous	---
l(1)-8	Heterozygous	---

[F] means the stock has been successfully frozen down in embryo state.

Number in brackets is number of embryos frozen down. 500 embryos usually constitutes a frozen line, but in cases of poor reproductivity or freezing embryos from heterozygous parents, 1000 is desired.

Using Inversions to Ascertain Recessive Lethals and Detrimentals

Having produced inversions, the next step was to utilize them in they way H. J. Muller used drosophila inversions to ascertain induced recessive lethals and detrimentals. Crossing over within the inverted segment in inversion heterozygotes leads to an anaphase bridge which in turn leads to death of the meiotic product. Thus inversion heterozygotes usually do not have offspring whose genetic material within the inverted segment (or immediately outside) has recombined. Thus the inversion acts as a single allele of relatively enormous genomic length. The appropriate use of these inversions with genetic markers located in and around them should mark a single large segment of the genome that could be studied for damage in its entirety. This should be a major advance over specific locus mutation systems where only mutations or deletions in the locus area could be ascertained. If an inversion system acts properly, any recessive change within the entire segment should be ascertained.

Considering several inversions and the markers available around them we picked inversion In(1)1Rk, covering a major portion of proximal chromosome 1 in the mouse. In an around this inversion were highly suitable markers that could be used in a 3 generation test to ascertain recessive lethals. Figure 1 shows the first mutation test system through which we produced several lethals. The system utilized the loci Splotch and leaden in the normal chromosome and necessitated mutagenizing the inverted chromosome. It was highly successful in producing lethals although at rates lower than anticipated.

Table 3 summarizes the lethals discovered and the cumulative totals of the genetic classes in the third generation. It is important to note the consistency over time of these totals indicating the genetic system has remained constant. These data were collected as part of the ongoing process of maintaining the lethal lines which are still extant as caged animals on the shelf. The lethals named "l(1)1RK through l(1)8Rk were all induced using In(1)1Rk in the first test system. Lethal l(1)9Rk was induced using the new mutagen test system. Lethal l(7)1Rk was induced using an inversion in chromosome 7, and thus the genetic classes involve a locus (Hbb) of that chromosome. For lethals on Chr 1, the ratios are of the Splotch phenotype to the normal phenotype in the 3rd generation. These ratios should be about 2:1 (actually 1.5:1 because of the partial prenatal mortality of the splotch carrying embryos), if no lethal or recessive detrimental were induced. In these cases all the cumulative totals are significantly different from 2:1. Those stocks which occasionally bear a wildtype (+) animal are in the inversion in a position that permits 2 or 3 strand double crossing over on either side of the lethal point. This is more likely in the center of the inverted segment. Those stocks with no wild type offspring produced are more likely nearer the breakpoints of the inversion, but within the inverted segment.
lethals which occasionally

Table 3 Lethal List and Third-Generation-Test Cumulative totals

Lethal No.	Male No.	1981 Sp:+	1983 Sp:+	1986 Sp:+	1987 Sp:+	1989 Sp:+	1991 Sp:+	1993 Sp:+
l(1)1Rk	2862	1029:86	1606:133	2893:208	3290:235	3404:242	4024:271	4024:271
l(1)2Rk	319	530:4	783:5	1171:7	1207:9	1305:9	1769:11	1826:11
l(1)3Rk	2883		36:0	83:0	101:0	117:0	137:0	137:0
l(1)4Rk	765	85:0	169:0	256:0	285:0	318:0	343:0	343:0
l(1)5Rk	959	629:7	942:8	1344:11	1414:11	1457:11	1986:13	2052:14
l(1)6Rk	1296	246:1	412:2	806:5	976:5	1059:5	1419:13	1464:13
l(1)7Rk	1312		314:1	604:2	624:4	628:2	909:3	909:3
l(1)8Rk	486						289:2	323:2
l(1)9Rk	92						21:1	35:1
				Hbb s/s:s/d:d/d				
l(7)1Rk	115			153:349:1				

Fig. 1 First Mutation Test System Using In(1)1Rk

```
                                        (mutagen
                                         treated)
                                            /
                  +(Sp ln)              In(+ +)
P                ---------      X       ------
                  +(+  ln)              In(+ +)
                                  |
                                  |
                                  |
              ____________________________________
              In( + + )                   In(+ + )
              ---------                   --------
               +(Sp ln)                    +(+ ln)
                  .
                  .                       (alternate)
                  .

              In( + + )*                  +(Sp ln)
G1            ---------          X        --------
               +(Sp ln)          |         +(+  ln)
                                 |
                                 |
      ____________________________________________________
      In( + + )*    In( + + )*    +(Sp ln)     +(Sp ln)
G2    ---------     ---------     --------     --------
       +(Sp ln)      +( + ln)     +(Sp ln)     +( + ln)

      (backcross     (discard)      (die)       (discard)
       or mate
      inter se)
          |
          |
          |
      ____________________________________
      In( + + )*    In( + + )*    +(Sp ln)
G3    ---------     ---------     --------
      In( + + )*     +(Sp ln)     +(Sp ln)

     (test class)                   (die)

          1/3           2/3
```

'Sp' is a dominant spotting marker, homozygous lethal in utero.
'ln' is a recessive coat dilution. Both genes are within the segment spanned by In(1)1Rk.

* signifies the presence of a presumed recessive; the absence of the test class in G3, or a consistent phenotypic effect in this class indicates that a recessive lethal or recessive detrimental has been induced.

Initial mapping of recessive mutants can be done effectively now using both systems employing In(1)1Rk. The old system (Fig. 1) in which 8 lethals were induced on Chromosome 1 necessitated mutagenizing the inversion carrying chromosome, (the obverse of that for the new system shown in Fig. 2.) Since the lethals in this case are within the inverted segment itself mapping them would be more difficult without genetic polymorphisms also within the inverted segment. Yet, the major reason we work with inversions is that they usually do retain their genetic integrity. On rare occasions, however, when In(1)1Rk has permitted a 2- or 3-strand double crossover to occur, we have been able to develop stocks with the inversion but carrying different alleles within it. These rare events have been picked up as variants in the test generation of our lethal induction work. The normal allelic arrangement within In(1)1Rk and the new variant chromosomes with In(1)1Rk are given below:

Chromosome	Inversion	Idh-1	Sp	ln	Pep-3	lethal
In(1)1Rk	In	b	+	+	b	+
In1Rk with lethals	In	b	+	+	b	l
Var. 1 of In1Rk	In	b	Sp	ln	b	+
Var. 2 of In1Rk	In	a	Sp	ln	b	+
Var. 3 of In1Rk	In	a	Sp	ln	b	+

Using variant 2 or 3 , we set up a unique protocol to map lethals within the inversion. The mating is as follows where the symbol order is Idh-1, Splotch, leaden, Pep-3, lethal. All but the lethal position are in correct locus order

	Parent No. 1		Parent No. 2
Chromosome A.	+(a + ln a +)		In(a Sp ln b +)
		X	
Chromosome B.	In(b + + b l)		In(b + + b l)

Both parental genotypes can be easily constructed from our present stocks. Parent No. 2 is homozygous for the inversion but heterozygous for Idh-1, leaden (ln) and a given lethal (l). This is the parent from which recombination will be ascertained. Parent No. 1 is heterozygous for the inversion, thus preventing recombination. One quarter of the offspring will die because of homozygous lethality. One quarter will be homozygous ln/ln which will be discarded. A combination of Chr. A of par. 1 and Chr. B of par. 2 (another 1/4 of the offspring) will confound our analysis, so we will have to determine for all IDH-1AB animals whether they are also PEP-3AB. If so they will be of this class and will be discarded from the analysis. Thus the 1/4 that remain will all involve only Chr. A of Par. 2 and Chr. B of Par. 1. From the phenotypes in this cross given below, all recombination classes classes and a parental class can be ascertained, using only Idh-1 and Splotch phenotypes

They are:

	Idh-1,	Sp,	lethal
Parental	a	Sp	+
	b	+	l
Recombination between Sp and [Idh, l]	a	+	+
	b	+	l
Recombination between Idh and [Sp, l]	b	Sp	+
	b	+	l
Recombination between l and [Sp, Idh]	b	+	+
	b	+	l

A direct counting of these classes gives an order to the three loci and an estimate of their distances unencumbered by inversion effects. Fig. 3 shows a map of the location of these lethals with respect to inversion In(1)1Rk.

If we construct the systems so that we give the mutagen to the non-inversion carrying chromosome (Fig 2), then the formation of linkage testing systems for the lethals will be easier, for we can manipulate the non-inverted non-lethal carrying chromosome more easily, i.e. we can put in any polymorphic markers we want. For this analysis we used Pep-3 with Idh-1 which makes things simpler, but the method is analogous. If we mutagenize the inversion-carrying chromosome, we need to wait for double recombination to provide the genetic heterogeneity within the inversion to do standard mapping tests of lethals.

For the lethals on Chromosome 1, we mated each lethal bearing animal with the other markers within the inversion to see if we have induced lethals at any specific locus. Also we mated the lethal bearing mice inter-se to ascertain that they are not overlapping each other. Full complementation was observed for all matings and no overlap of standard markers were observed for any of the lethals, except lethal-3 which overlaps the leaden (ln) locus.

Fig. 2 . New Mutation Test System Using a Variant of In(1)1Rk

```
                                        (mutagen
                                         treated)
                                            /
                  In(Sp ln)             +(+ +)
P                 ---------     X       ------
                  In(+  ln)     |       +(+ +)
                                |
                                |
              ____________________________________
               +( + + )                  +(+ + )
              ---------                 --------
              In(Sp ln)                 In(+ ln)
                  .
                  .                    (alternate)
                  .
               +( + + )*                In(Sp ln)
G1            ---------        X        ---------
              In(Sp ln)        |        In(+  ln)
                               |
                               |
     ____________________________ ____________________________
      +( + + )*     +( + + )*     In(Sp ln)     In(Sp ln)
G2   ----------    ----------     ---------     ---------
     In(Sp ln)     In( + ln)      In(Sp ln)     In( + ln)

     (backcross     (discard)       (die)        (discard)
       or mate
      inter se)
          |
          |
          |
      ____________________________________
      +( + + )*     +( + + )*     In(Sp ln)
G3   ---------    ----------     ---------
      +( + + )*    In(Sp ln)      In(Sp ln)

     (test class)                   (die)

         1/3           2/3
```

'Sp' is a dominant spotting marker, homozygous lethal in utero.
'ln' is a recessive coat dilution. Both genes are within the segment spanned by In(1)1Rk.

* signifies the presence of a presumed recessive; the absence of the test class in G3, or a consistent phenotypic effect in this class indicates that a recessive lethal or recessive detrimental has been induced.

Fig. 3 The map location of lethals induced with In(1)1Rk

```
                    Chromosome 1
                         cM
                          0 - Centromere

                          2

                          4

                          6 - fz
                   |-----50
                   |      49 - Pep-3
                   |      48
                   |
                   |      46 -----------|
                   |                    |
                   |      44            |(1-7)
                   |                    |
                   |      42            |
                   |         - ln (1-3) |
                   |      40 -----------|
                   |
                   |      38
                   |
                   |      36 - Sp
                   |
                   |      34
                   |
                   |      32 ----(1-2)
                   |
                   |      30
                   |
                   |
                   |      27 - Idh-1
In(1)1Rk           |      26
                   |
                   |
                   |
                   |      22
                   |      21 -----------|
                   |                    |
                   |                    |
                   |      18            |
                   |                    | (1-1)
                   |                    |
                   |                    |
                   |      14            |
                   |                    |
                   |                    |
                   |      11 -----------|
                   |      10
                   |
                   |       8
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                          51


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Reproductive effects of inversion in various combinations.

We have combined inversions on the same chromosome in doubly-heterozygous condition in the same animals for a variety of purposes. From studies of 35 such combinations, we can make the following generalizations:

1. Where two inverted segments lie outside each other,

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there is no effect on fertility on males and little effect on females.

2. Where one inverted segment lies wholly within the other,

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there is no apparent effect on fertility in either males or females

3. Where two inverted segments have single overlapping breakpoints

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the female has reduced litter size but is fertile, whereas the male is sterile and invariably has a very small testis, where we think development is stopped at the pachytene stage of meiosis. The females in these cases show varying percentages of deciduomas in their uterine contents, and different timing of embryonic mortality, which seems however to be consistent for the particular inversions pair. The explanations of these effects are interesting to explore in themselves, and these various combinations of inversions afford another route for producing developmental mutants, that can be consistently reproduced and studied.

4. Where two inverted segments are nearly but not quite the same,

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they usually adjust in pairing, probably by permitting a little non-homologous pairing or juxtaposition (i.e. seem to overlook their differences), show slightly elevated frequency of first meiotic anaphase bridges (where they fail to completely overlook their differences), and have only slightly reduced litter size. These situations need to be explored further for developmental mutants, since the genetic recombination effect, i.e. concomitant partial trisomy and partial monosomy, is minimal and lethality would

be due to more specific and smaller regions of a chromosome, containing fewer genes. The problem with embryonic lethals produced by translocations for tools in developmental research is that the partial trisomies and partial monosomes are so extreme that the possibility of study of specific gene effects is very remote.

Timing of lethality and nature of the developmental effects

In collaboration with Dr. D.M.Juriloff, post-doctoral associate, (Juriloff et al. 1985) and Dr. Sen-ichi Oda, a visiting scientist, (Oda and Roderick, 1993, in preparation) we studied the timing and nature of seven of these lethals and three of the inversions that are homozygous lethal. Probably these lethals are deletions and cover more than one locus. The timing of lethality varies. Of the 10, one was a preimplantation lethal, two revealed early post-implantation deaths around days 4 to 5, two showed somewhat later deaths around days 6 to 7 and the remaining five showed death occurring between days 8 to just after birth. Of the five characterized by late death, all showed unique syndromes and all showed more early deaths (deciduomas) as well. The five syndromes were: Lethal-1 was characterized by otocephaly detectable as early as 11 days, with some variation in severity (Juriloff et al. 1985). Animals homozygous for lethal-2 showed smooth edematous tight skin at birth. There appears to be reduced proliferation of dermal cells and hair follicles. Lethal-7 animals showed open eye lids, omphalocele, and a slender face. The nasal pits were close together. Lethal-8 animals showed polydactyly, some syndactyly, a screw tail, and micrognathia, micro- or anophthalmia. Animals homozygous for In(15)21Rk showed omphalocele, syn- or oligodactyly, and open eyelids. This collection of independently induced recessive lethals may provide estimates of the general nature and extent of the embryonic burden of recessive lethals in mammals. The important aspect here is that all lethals are marked by associated markers that are not necessarily compounded in the test animals. Further the lethals can be studied for timing and nature of effect, and that so far each lethal appears to have a separate constellation of effects. And further, the general map location of each is known, and can be greatly refined.

Prospects for future work:

Although we have met the objectives of the proposed work, much basic genomic research could be accomplished utilizing this invaluable set of inversions and lethals.

1. Further fine structure analysis of the inversions is important for many aspects of their continued use in mutagenesis and other biological studies. Now with the microsatellite markers, it would be possible to map them with good precision. This technology is widely available. It would need to be associated with the ability to recognize inversion heterozygotes weither through athe ascertainment of meiotic anaphase bridges or through karytype analysis. The former would be far less expensive in supplies and man-power, but is a technology that only our laboratory has consistently utilized effectively.

2. For the most part the length of the inversion as finally determined by karyotypic and linkage analysis was highly correlated with the frequency of first meiotic anaphase bridges. But some inversions clearly did not show this association. In all cases where there was an exception, the bridge count was much lower than expected. It is possible that other aberrations were induced simultaneously in or around the inverted segment that made pairing within the inversion less probable, thus lowering the opportunity for crossing over and thus lowering the frequency of anaphase bridges. Also it is possible that these inversions may be an argument for the hypothesis proposed by Dr. Terry Ashley; she has proposed that whether breakage occurs in a light or dark band will have a bearing on the subsequent pairing process, specifically whether or not homologous or non-homologous pairing will occur contiguous to the break. Dr. Ashley has imported our inversions to her laboratory for study.

3. At the end of the project we had begun synaptonemal complex analysis of inversion heterozygotes comparing presence of loops with the nature and length of the inversion. An initial finding which will be completed for publication is that the very long inversions do not show the typical inversion loop but instead undergo reverse pairing where the centomeric ends are left unpaired. We are using our initial data to determine what length of inversion is more likely to show reverse pairing. We suspect it depends on the proportion of the chromosome inverted rather than the length alone.

4. Further work would be useful on the study of the developmental timing of lethality of the induced lethals and the inversions that are homozygous lethal. This system can ascertain pre-implantation lethals as well as post-implantation lethals at all stages up through birth. It is the only one we are aware of where this broad spectrum can be ascertained easily in a single test.

5. We have evidence in specific cases that crossing over immediately outside the inversion segment is increased in inversion heterozygous males. The extent to which this universally applies to mouse inversions should be evaluated and the extent to which this occurs relevant to chromosome size and proportion of inverted segment to chromosomal length should be ascertained.

6. Perhaps most important would be further work utilizing the new

lethal test system that has been produced. It has significant advantages over presently used whole body mutation test systems. We estimate that it will detect the induction of mutations in about 5% of the mammalian genome, which is the distance covered by the inversion and a small region outside the inversion also under the effect of "reduced" recombination. Furthermore the lethals induced can be maintained and studied for developmental effects, timing of lethality, and effective burden on the reproductive performance of the mouse. This test is simple in that it involves only typing animals from easily discernible exterior markers. It can be done by animal caretakers who are careful record keepers. No equipment is necessary. The test deserves broad testing and actual use in ascertaining recessive lethals and detrimentals in a large facility.

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