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MASTER

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OBJECTIVE

The purpose of this project is to produce improved mutagen-testing systems in mice. Our approach is to produce chromosomal inversion systems and to improve the techniques necessary to induce, detect, genetically define, and combine inversions in effective useful mutation-test systems. Another specific objective has been to test the systems produced with respect to their effectiveness. Another objective has been to mark, maintain, and study recessive detrimental and lethals induced in the validation-testing of these systems. Of particular importance is to study induced recessives for dominant effects on fitness. A final broad objective has been to use the induced inversions and recessive lethals for studies of basic problems in mammalian genetics, growth and development.

SCOPE OF RESEARCH

The inversions, Robertsonians, and translocations induced need to be genetically and cytologically mapped precisely so that specific mutation-test systems can be constructed. Then useful combinations of these need to be constructed and then validated in a mutation test assay. In addition to the practical need for mutation-test systems which these aberrations afford, it is important to study the nature and effects of the inversions themselves, especially in meiosis, so that problems in their use can be identified and avoided. At the present time, the idiosyncrasies of mammalian inversions, such as absence of reduced reproductive performance, have added to their value in the objectives of this program.

MAIN RESEARCH ACCOMPLISHMENTS

Inversions produced. We induce and detect inversions by looking for first meiotic anaphase bridges in one testis of sons of males treated with radiation or a chemical mutagen. The natural frequency of bridges at this division is about 5 per cent, making it necessary to search for animals which display a frequency significantly in excess of 5 per cent. These inversions are all paracentric inversions, since anaphase bridges are not characteristic of pericentric inversions. We also need to induce and study pericentric inversions and thus another method was established to use simultaneously. The chromosomes of the mouse are all acrocentric providing little probability of inducing pericentric inversions, since breaks would need to occur on both sides of the centromere. Therefore we have given triethylene melamine (TEM) as a mutagen to mice carrying at least three Robertsonian chromosomes and examined the banded karyotypes of sons and daughters for presence of pericentric inversions in these chromosomes.

These methods continue to be useful in inducing inversions. Table 1 shows all the inversions and presumptive inversions produced in our laboratory to date. The table shows the different lengths of the inversions (indicated by their characteristic bridge frequency), the various chromosomes affected, and the variety of possible physiological decrements associated with inversions. The last three were discovered this year, two of which were apparently induced by TEM and the other apparently occurring spontaneously. The POS lines in which these inversions occurred were derived originally from a cross of mouse stocks of the Poschiavinus Valley by a standard laboratory stock. The Poschiavinus

stocks are one of the few natural populations of mice carrying Robertsonian meta-centrics which are themselves useful in our work as described elsewhere. Inversion In33Rk has appeared in animals with no ancestral experimental treatment with a mutagen. This spontaneous occurring inversion may exist in the original Poschiavinus populations, or it may have occurred in our laboratory during maintenance of these stocks.

Figure 1 shows the known linkage relationships of all inversions induced or discovered to date in the mouse. Two of those shown on the map were discovered at Harwell, England. The inversions cover an appreciable portion of the genome, and variable portions of various chromosomes. Inversion systems for assaying mutagenesis can now be constructed in a variety of ways with various explicitly chosen advantages.

Cytology of inversions. In(11.13LD)29Rk was confirmed to be a pericentric inversion by synaptonemal complex studies (in collaboration with Dr. M. J. Moses at Duke). Although the original female carrier had only three offspring and the carrier son never produced offspring, it was not obvious that reduced fertility was the direct result of the inversion. Testicular preparations from the male appeared normal with respect to presence of meiotic cells. The female was also heterozygous for three Robertsonian chromosomes which are known to reduce fertility.

Reassessment of the cytological breakpoints of In(2)5Rk show that inversion to be much more distal than previous cytological studies indicated, with breakpoints in bands 2D and 2H1. This position is consistent with that indicated by genetic and synaptonemal complex data.

Synaptonemal complex studies of In(1)1Rk/In(1)23Rk double heterozygotes show no discrepancies in pairing with the pachytene bivalent appearing as a normal uninverted bivalent. This confirms our suspicion that In1 and In23 are the same inversion.

We have reported previously that In11Rk was not detectable cytologically on Chromosome (Chr) 12. It has now been shown by our laboratory and others that the Chr 12 genes are really located on Chr 3. We have tentatively identified In(3)11Rk cytologically now and more precise cytological studies are in progress.

Translocations. Of the translocations induced during our program to produce inversions, 5 have been propagated and are maintained in our colonies as new chromosomal markers. These are T(1;13)2Rk (only viable in conjunction with In(1)24Rk), T(5;8)3Rk, T(12;17)4Rk, T(3;4)5Rk, and T(10.14)8aRk. T(3;4)5Rk was used to demonstrate that ma and Va, previously assigned to Chr 12, are actually located on Chr 3.

TABLE 1. INVERSIONS INDUCED IN THE MOUSE.

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Animal number	Inversion symbol	Anaphase bridge frequency	Induced* by	Induced** on	Comments
471		.379 ± .033	800 R	Ra, Sd	Functionally sterile
587		.176 ± .034	"	DBA/2J	Died before mating
618	In(1)1Rk	.340 ± .008	900 R	"	
639		.151 ± .026	"	"	Fertile, discarded
816	In(5)2Rk	.192 ± .010	850 R	"	
2414		.340 ± .048	900 R	"	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	"	"	
3563	In(10)6Rk	.262 ± .018	"	"	
4587	In7Rk	.150 ± .024	800 R	"	Discarded
4609	In8Rk	.179 ± .019	"	"	Lost
5204	In(5)9Rk	.737 ± .020	TEM .2	C3D2F ₁	
6567	In(1)10Rk	.741 ± .033	EMS	"	Functionally sterile
6845	In(3)11Rk	.294 ± .055	TEM .2	C57BL/6J	
6857	In(1)12Rk	.228 ± .047	"	"	
7877	In(7)13Rk	.447 ± .040	"	C3D2F ₁	
8677	In14Rk	.347 ± .043	"	DBA/2J	
10434	In(3)15Rk	.689 ± .038	"	B6D2F ₁	Functionally sterile
11318	In16Rk	.458 ± .059	TEM .3	DBA/2J	T(1;In8)1Rk, sterile
11323	In(10)17Rk	.648 ± .040	"	"	Small homozygotes
11449	In(15)18Rk	.366 ± .024	"	"	
11675	In(2)19Rk	.609 ± .035	"	"	
11684	In(11)20Rk	.473 ± .037	"	"	Litter size reduced
11865	In(15)21Rk	.469 ± .032	TEM .4	"	Possibly semi-sterile
11951	In(14)22Rk	.663 ± .036	"	"	
12164	In(1)23Rk	.364 ± .040	"	"	Probably In(1)1Rk
12174	In(1)24Rk	.728 ± .046	"	"	
12452	In(12)25Rk	.459 ± .048	"	"	
12953	In(9)26Rk	.237 ± .028	"	"	Identified cytologically
16567	In27Rk	.500 ± .067	TEM .3	POS (M)	Died before mating
16647	In28Rk	.559 ± .085	"	POS (AxD)	
(7780IJ)	In(11-13LS)29Rk ***		"	POS (M)	Identified cytologically
21716	In(5)30Rk	.270 ± .047	TEM .4	POS (F)	
21923	In31Rk	.700 ± .102	TEM .3	"	
25022	In32Rk	.143 ± .054	TEM .5	" (P)	
25086	In33Rk	.614 ± .030	Spontaneous	" (M)	
25094	In34Rk	.417 ± .064	TEM .5	" (P)	

Footnotes, next page.

Footnotes, TABLE 1.

* Numbers by TEM 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 first meiotic anaphase bridges.

Inversion symbol: 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 is the symbol of our laboratory.

FIG. 1. Inversion Linkage Map of the Mouse.

Each inversion is denoted beside the segment it encompasses. The straight line indicates the portion of the segment for which "allelism" with genetic markers has been ascertained. The wavy portion depicts that area to which the inversion extends based on cytological evidence. The dotted lines indicate that the inversion, because of its high bridge frequency may extend further.

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Robertsonian chromosomes. The importation of Robertsonian chromosome-carrying mice from Dr. Alfred Gropp's laboratory is now complete and all the stocks are in our MGL (high-level isolation) mouseroom. We are in the process of producing stocks with homozygous Robertsonian combinations for ease of maintenance and to use in inducing new inversions. We now have available in our colony at least one Robertsonian chromosome involving each of the 19 chromosomes of the mouse. As they become homozygous in useful combinations we will begin to treat them in our TEM treatment-screening program to induce new inversions in Robertsonian chromosomes.

Mutagen test systems. We have continued to validate the inversion test system using In(1)1Rk. In addition we are now ready to begin inducing lethals in the double inversion system utilizing In(1)1Rk and In(7)13Rk. This system will more than double the proportion of the genome to be assayed although we expect a slightly less efficiency per breeding pen in the last generation due to the greater number of classes of animals to be raised. We will continue to use Sp (Splotch) and ln (leaden) in testing for lethals on Chr 1, but will use two good isozyme markers from blood tissue, Gpi-1, and Hbb for assessing lethals induced in Chr 7. The Splotch-leaden stock we already have used has the right allelic differences at these loci on Chr 7 to make it useful to breed with the double-inversion stock.

The double inversion stock is now being used to assess lethal mutations in wild populations. This work is being done in collaboration with Dr. Michael Petras of the University of Windsor, Windsor, Ontario, Canada. Dr. Petras, a biochemical and population geneticist spent several weeks in our laboratory this year working out biochemical techniques and preparing for the testing of this inversion system on wild populations.

Combining Robertsonian chromosomes and inversions is also important in increasing the portion of the genome to be screened for newly induced recessive mutations. We have successfully incorporated In(1)12Rk into Rb(1.3)1Bnr through crossing over in an animal doubly heterozygous for both chromosomal aberrations. Heterozygotes are now mated and we are screening offspring using blood cultures to find homozygotes.

Meiotic pairing. We have continued our collaboration with Dr. Montrose J. Moses of Duke University studying the synaptonemal complex in inversion heterozygotes. An initial paper on this work is in preparation.