C00-4159-3

GENETIC AND DEVELOPMENTAL STUDY OF A COMPLEX LOCUS IN THE HOUSE MOUSE

Progress Report

for period August 1, 1976 - July 31, 1977

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June 1977

Prepared for

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

Under Contract No. EY-76-S-02-4159

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Abstract of Progress Report

We have maintained and studied certain aspects of the genetics and embryology of approximately 40 chromosome 17 mutations in the mouse, including eight newly derived t-haplotypes. Two dominant T mutations (T^{Hp} and T^{Orl}) have been characterized as having a homozygous lethal phenotype different from T; they die earlier in development, at 6-7 days with defects of the embryonic ectoderm. The same mutations, which are both probably chromosome deletions produce mild runting in heterozygous condition, and more severe runting in compound with all t-haplotypes that have been studied. Attempts to map the position of a recessive viable allele t^{38}, have given results that suggest that t^{38} is not a point mutation, but may extend over a distance of 3 centimorgans. Data from the same set of experiments indicate that particular combinations of mutations in females may result in gametic selection, i.e., the preferential selection by the egg of one of the two classes of sperm from heterozygous males. New experiments designed to analyze the relationship between t-haplotypes and H-2 type in wild mice are in progress.

PROGRESS REPORT

Principal Investigator:

Dorothea Bennett, Ph.D. 10% of time (no change anticipated). Contract requirements have been complied with.

Numbers (1) - (6) below refer to specific items in the project abstract submitted for the current contract year. Item (7) refers to work that was not anticipated at that time, and is justified in the project abstract for the coming year's contract proposal.

(1) Stock Maintenance and Supply

I. The following mutations at the T/t locus are maintained:

Complementation Group	Mutations
T	T, T^{10r}, T^{20r}, T^{30r}, T^{60r}, T^{Hp}, T^{0r1}
t^0	t^0, t^1
t^9	t^{w18}
t^{12}	t^{12}, t^{w32}
t^{w1}	t^{w1}, t^{w12}, t^{w71}
t^{w5}	t^{w5}, t^{w75}, t^{w93}, t^{w94}
t^{w73}	t^{w73}
$t^{semilethal}$	t^{w2}, t^{w8}, t^{w97}
t^{viable}	t^{38}, t^{46}, t^{52}, t^{w82}, t^{w84}, t^{w86}, t^{w88}, t^{w90}, t^{w92}, t^{w95}, t^{w96}

II. The following chromosome 17 markers or mutations are maintained:

Ki, tf, gk, T190, T138.

III. We have supplied the following laboratories with T/t mutations:

Dr. James Archer
Searle and Company
England

Dr. Arnheim
CUNY
Stonybrook, New York

Dr. Ann Baker
Rockefeller University
New York, New York

Dr. Charles Epstein
University of California
San Francisco, California

Dr. Alfred Gropp
Lubeck, West Germany

Dr. Craig Hammerberg
Johns Hopkins University
Baltimore, Maryland

Dr. Nina Hillman
Temple University
Philadelphia, Pennsylvania

Dr. Francois Jacob
Institut Pasteur
Paris, France

Dr. Jan Klein
University of Texas
Dallas, Texas

Dr. Gloria Koo
Sloan-Kettering Institute
New York, New York

Dr. Joe Larry
University of Alabama
University, Alabama

Dr. Hugh O. McDevitt
Stanford University
Stanford, California

Dr. Russell Pollard
Centre for Disease Control
Ontario, Canada

Dr. Mike Sherman
Roche Institute
Nutley, New Jersey

Dr. Sid Strickland
Rockefeller University
New York, New York

(2) Embryology of T^{Hp} and T^{Orl} homozygotes

Embryological studies of lethal homozygotes of $\underline{T^{Hp}}$ and $\underline{T^{Orl}}$ demonstrate that both die several days earlier than $\underline{T}$ homozygotes. When examined histologically at 6 days gestation, $\underline{T^{Orl}}$ homozygotes are small distorted egg cylinders with extreme pycnosis in the embryonic ectoderm. $\underline{THp/THp}$ embryos at the same age show the same types of abnormality, but to a lesser degree. Both types of embryos have degenerated almost entirely by seven days of gestation; their decidual capsules enclose a large implantation site that includes a well formed ectoplacental cone and shell of trophectoderm with giant cells, but the embryo proper is either lacking or present only as a small knob of dead pycnotic cells. Our conclusion is that inner cell mass cells are selectively killed by homozygosity for either of these genes.

(3) Deleterious interactions of T^{Hp} and T^{Orl} with recessive t-haplotypes

Preliminary observations had indicated that $\underline{T^{Hp}}/+$ and $\underline{T^{Orl}}/+$ heterozygotes were small and somewhat runted compared to their +/+ littermates. Runting was still more obvious in $\underline{T^{Hp}}/t$ or $\underline{T^{Orl}}/t$ heterozygotes. Since both $\underline{THp}$ and $\underline{TOrl}$ are suspected on other grounds (chromosome cytology and illicitation of pseudodominance in a closely linked recessive marker--qk-) it seemed that the increased degree of abnormality seen when these dominant genes were combined with t-haplotypes (which have also been suspected of being chromosomal aberrations) might be due to overlapping deletions. This in turn meant that quantitating the abnormality in heterozygotes for various t-haplotypes might provide a way of size-mapping t-mutations relative to one another.

Therefore, we set up experiments first to crudely quantitate the degree of runting in $\underline{T^{Hp}}/+$ and $\underline{T^{Orl}}/+$ heterozygotes. Litters containing such animals were counted and weighed within one day of birth; the average weight of T/+ and +/+ pups was determined; to eliminate variation due to litter size or age the ratio: $\frac{\text{average weight of T/+ individual}}{\text{average weight of +/+ individual}}$ was calculated.

For $\underline{T^{Orl}}$ we scored 146 +/+ animals and calculated an average weight of 1.8 g; whereas 220 $\underline{TOrl}/+$ littermates had an average weight of 1.6 g, giving a ratio of 0.9.

For $\underline{T^{Hp}}$ the average weight of 82 +/+ newborns was 1.9 g, and 75 $T^{Hp}/+$ littermates weighed 1.8 g on the average. The ratio was again 0.9

Crosses of $\underline{T^{Hp}}$ or $\underline{T^{Orl}}$ males with tailless females of different t-genotypes have been made, and the following results obtained on offspring evaluated at birth.

		NEWBORN OFFSPRING						
		+/+		T/+		T^{Hp}/t		Weight Ratio T^{Hp}/t: (+/+ + T/+)
♀	Genotype	#	Average Weight	#	Average Weight	#	Average Weight	
$T^{Hp}/+$ ♂	T/t^{w5}	44	1.9	40	1.9	27	1.3	0.7
	T/t^{1}	70	1.7	47	1.6	67	1.4	0.8
$T^{Orl}/+$ ♂	T/t^{w5}	29	1.8	25	1.9	18	1.7	0.9
	T/t^{1}	22	1.6	19	1.6	24	1.6	1.0
	T/t^{w1}	29	1.6	17	1.7	34	1.4	0.9

These data suggest strongly that $\underline{T^{Hp}}/t$ animals are at a disadvantage to both +/+ and T/+ in terms of birth-weight, although Chi-square analysis shows that they are produced in ratios that do not differ significantly from expected ($P > .05$ for 1:1:1 ratio in both crosses). $\underline{T^{Orl}}/t$ newborns appear, however, to be only marginally disadvantaged.

Further experiments to explore the interaction of T^{Orl} with t-haplotypes were done, this time using $T^{Orl}/+$ females and males of various t-haplotypes. The results are as follows:

	NEWBORN OFFSPRING						
	+/+		T/+		T^{Orl}/t		Weight Ratio
♂Genotype	#	Average Weight	#	Average Weight	#	Average Weight	T^{Orl}/t: (+/+ + T/+)
T/t^{1}	36	1.8	11	n.d.	26	1.3	0.7
T/t^{12}	26	1.4	2	n.d.	14	1.0	0.7
T/t^{w1}	36	1.8	4	1.9	16	1.3	0.7
T/t^{w12}	51	1.5	1	n.d.	68	1.2	0.8
T/t^{w71}	20	1.9	6	2.0	19	1.4	0.7
T/t^{w32}	27	1.6	2	n.d.	18	1.3	0.8
T/t^{w73}	18	1.6	2	n.d.	15	1.1	0.7
T/t^{s1}	49	1.6	16	1.9	54	1.3	0.8

In all cases T/t animals show a more pronounced deficit in weight than T/+ heterozygotes. However, there is no clear-cut difference between the various t-haplotypes tested; thus they may all overlap with the T^{Orl} defect to the same extent.

(4) Fine structure mapping of T/t region

Experiments reported last year that were designed to map inter-gene distances in $\frac{+ \quad Low \quad tf}{t^{38} \quad + \quad +}$ females gave anomalous results. Virtually all recombination occurred in the Low-tf interval, and almost none in the t38-Low region, although independent measurements of the same intervals in females using the marker T instead of t^{38} had given map distances of 0.05 for the T-Low interval but only 0.01 for the Low-tf interval. Since Low has some of the characteristics of a t-allele, we suspected that some kind of interaction might be responsible for the observed deficiency of crossing-over between Low and t^{38}.

Accordingly, we have repeated these experiments using another marker qk, which maps very close to Low. Females of genotype $\frac{+ \quad qk \quad tf}{t^{38} \quad + \quad +}$ were constructed and mated to THp tf/+ tf males. The pseudodominance of qk in the presence of THp permits its immediate detection. The genotypes and phenotypes of informative progeny (short-tailed and tailless) are given below:

	genotype	phenotype	offspring recorded at weaning
non-recombinants	$\frac{+ \quad qk \quad tf}{THp \quad + \quad tf}$	short-tailed tufted, quaking	80
	$\frac{t^{38} \quad + \quad +}{THp \quad + \quad tf}$	tailless non-quaking non-tufted	50
recombinant in t^{38} - qk region	$\frac{+ \quad + \quad +}{THp \quad + \quad tf}$	short-tailed non-tufted non-quaking	0
	$\frac{t^{38} \quad qk \quad tf}{THp \quad + \quad tf}$	tailless quaking tufted	0
recombinant in qk - tf region	$\frac{+ \quad qk \quad +}{THp \quad + \quad tf}$	short-tailed quaking non-tufted	4
	$\frac{t^{38} \quad + \quad tf}{THp \quad + \quad tf}$	tailless non-quaking tufted	3

These data give an overall recombination fraction for the t^{38} - tf region of 0.05, which is quite compatible with the data mentioned above for the T - tf interval. However, all of the recombination has occurred between qk and tf, and none between t^{38} and qk, although again independent evidence has shown T and qk to be about 0.03 units apart. The conclusion we must draw from this is that t^{38} which acts as a true genetic allele of T also appears to be allelic to qk some distance away. Thus, either t^{38} is itself a recombination suppressor (for which there is no evidence) or the mutation occupies a region of at least 3 centimorgans.

These experiments also produced interesting data of another kind. The total progeny scored at birth deviated significantly from Mendelian expectations, as follows:

	proportion expected	observed numbers	observed proportion
normal-tailed	2	352	2.8
short-tailed	1	128	1.0
tailless	1	100	0.8

The Chi-square here is 28.1, with $P < 0.001$. The deficiency of short and tailless progeny at birth is not explainable by litter size, and in fact the data presented above in section (3) show that segregation ratios at birth are normal in matings of $T^{Hp}/+$ males by T/t females. These data may reflect some characteristic peculiar to eggs from $\frac{+ \; qk \; tf}{t^{38} \; + \; +}$ mothers that leads to differential fertilization by sperm from $\frac{}{THp}$ heterozygotes.

(5) Conversion of stocks to T qk tf/t

About 50% of our mutant t-stocks are now being carried with the T qk tf chromosome. Especially in light of the results presented in (4) above, we think this will be an important way of defining the origin of newly derived t-mutations.

(6) Analysis of new t-haplotypes

Complete or partial genetic analysis has been carried out for eight newly arisen t-alleles in our colony. All of these have arisen from a pre-existing lethal or semilethal mutation by abnormal recombinational events, and all are known to produce viable homozygotes. The mutations that we have analyzed in the past year, and their characteristics, are given in the table below:

Allele	Parent Allele	t/t Relative Viability	Transmission Ratio of t	Male t/t Fertility
t^{51}	t^{0}	viable	i.d.	i.d.
t^{52}	t^{12}	viable	i.d.	normal
t^{w89}	t^{w32}	viable	i.d.	normal
t^{w90}	t^{w5}	low?	i.d.	normal
t^{w91}	t^{w5}	viable	69%	normal
t^{w92}	t^{w18}	viable	i.d.	normal
t^{w95}	t^{w1}	viable	i.d.	normal
t^{w96}	t^{w1}	viable	i.d.	i.d.

Preliminary tests are underway for an additional 12 variants newly arisen in the past year.

(7) Trapping and genetic analysis of wild mice

Samples of wild mice were obtained from wild populations in several different areas, and are being analyzed for: (1) presence of t-haplotypes (2) complementation group of t-haplotype, if present; (3) t-transmission ratio, and (4) H-2 type. The data we have so far are as follows:

Source	t-haplotype?	Haplotype Designation	Transmission Ratio	Complementation Tests[1] t^0	t^9	t^{12}	t^{w1}	t^{w5}	t^{w73}	t^{w93}	t^{w94}	H-2[2] Type
Dutchess County: Decker Farm	lethal	t^{w93}	1.0	n.d.	6/6	n.d.	8/13	0/48	8/17	-	0/10	t^{w5}
Dutchess County: Messerick Farm	lethal	t^{w94}	.99	n.d.	6/3	0/10	7/13	0/33	11/14	0/17	-	t^{w5}
Dutchess County: Whalen Farm	lethal	t^{w97}	1.0	n.d.	n.d.	16/8	0/33	0/22	n.d.	n.d.	n.d.	n.d.
Dutchess County: Rossway Poultry Farm	semi-lethal	t^{wB}										n.d.
Dutchess County: Dianto Farm	No											n.d.
Dutchess County: Shields Poultry Farm	No											n.d.
Amsterdam, Holland: Zoo	No											n.d.
Amsterdam, Holland: Zoo	No											n.d.
Amsterdam, Holland: Zoo	No											n.d.
Dutchess County: Pierson Farm	Yes	t^{wC}										n.d.
Israel	Yes	t^B										n.d.
Israel	Yes	t^F										n.d.
Israel	Yes	t^0										n.d.
Israel	Yes	t^N										n.d.

Footnotes to table

(1) Numbers given, e.g., 6nt/24ot, indicate that matings of tailless T/t^{w93} x tailless T/t^{0} animals produced a total of 6 normal-tailed (=nt) (t^{w93}/t^{0}) animals and 24 tailless (=ot) (T/t^{w93} or T/t^{0}) animals, thus indicating complementation of the two lethal haplotypes t^{w73} and t^{0}. Tests (see boxes) where no normal-tailed offspring are produced indicate that the two haplotypes are in the same complementation groups. So far, the three wild haplotypes that we have tested all fall in the t^{w5} group, which is by far the most common haplotype found in wild mice.

(2) H-2 typing of these mice is being done by Jan Klein at the Southwestern Medical School, Dallas. The two lines so far typed by Klein have the same H-2 type found in the prototype t^{w5} haplotype and the one other (t^{w75}) member of that complementation group that has been tested.

Preprints + Reprints Removed

Publications

Hammerberg, C., Klein, J., Artzt, K., and Bennett, D. (1976) Histocompatibility-2 System in Wild Mice. II. H-2 Haplotypes of t-bearing mice. Transplantation 21: 119-212. (Six reprints enclosed; submitted as document #COO-2497-04).

Bennett, D., Dunn, L. C., and Artzt, K. (1976) Genetic change in mutations at the T/t locus. Genetics 83: 361-372. (Six reprints enclosed; submitted as document #COO-2497-05).

Spiegelman, M., Artzt, K., and Bennett, D. (1976) Embryological study of a T/t locus mutation (t^{w73}) affecting trophectoderm development. J. Embryol. Exp. Morph. 36: 373-381. (Six reprints enclosed; submitted as document #COO-2497-06).

Bennett, D. Genetically programmed abnormalities of cell interactions. In: Proc. 1st Int. Symp. on The Molecular Basis of Cell-Cell Interaction. In press. (Submitted here as document #COO-4159-01).

Rittenhouse, E., Dunn, L. C., Cookingham, J., Calo, C., Spiegelman, M., Dooher, G. B., and Bennett, D. Cartilage matrix deficiency (cmd), a new autosomal recessive mutation in the mouse. J. Embryol. Exp. Morph., in press. (Submitted here as document #COO-4159-02).