Genet. Res., Camb. (1975), **24**, *pp.* 207–213
With 1 *text-figure*
Printed in Great Britain

Further observations on the hairpin-tail (T^{hp}) mutation in the mouse

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(*Received* 27 *June* 1974)

SUMMARY

The hairpin-tail allele (T^{hp}) of brachyury (T) on chromosome 17 of the mouse is unique in that the phenotype of heterozygous offspring depends on which parent contributed the T^{hp}. The present paper offers formal proof of this fact using the t^{h2} allele of T as a marker, and discusses possible modes of action of the hairpin allele.

1. INTRODUCTION

Hairpin-tail (T^{hp}) is an allele of brachyury (T) situated on chromosome 17 (linkage group IX) of the mouse (Johnson, 1974). It appears to differ from all previously described mammalian genes in that the phenotype and viability of the heterozygote depend on the parent from which the T^{hp} gene was inherited. Heterozygotes with a $T^{hp}/+$ father ($T^{hp}/+^{1}$) are short tailed and, in the main, viable. Heterozygotes whose T^{hp} was derived from the egg ($T^{hp}/+^{2}$) are short tailed, oedematous, often have postaxial polydactyly and invariably die *in utero*. That this is not a simple maternal effect may be demonstrated in crosses of $T^{hp}/+$ ♂♂ × $T^{hp}/+$ ♀♀: both types of heterozygote are found in the progeny. The present paper offers a formal proof that the T^{hp} gene in $T^{hp}/+^{1}$ and $T^{hp}/+^{2}$ heterozygotes from crosses between $T^{hp}/+$ parents is derived from the sperm and egg respectively, and discusses some possible mechanisms which may account for this unique effect.

Mutations so far isolated at the T locus fall into two groups:

(1) A short series of dominants (T^{x}) all producing short tails when heterozygous, lethal when homozygous and producing a compound (T/T^{x}) which is lethal *in utero* (T^{h}, Lyon (1959); T^{hg}, Kumineck (1959, 1960); T^{c}, Searle (1966).

(2) A much longer series of 75+ (Dunn, 1964) recessive alleles (t^{v}) which seem not to comply with the rules of classical genetics (see Gluecksohn-Waelsch & Erickson (1970) for review). Recessive t alleles interact with T in various ways, producing tailless mice or suppressing the effect of T to yield a normal tail. Hairpin-tail reacts with one such t allele, t^{h2} (Lyon & Meredith, 1964), in the same way as

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does T, to produce a tailless compound. However, data so far presented on the cross $T^{hp}/+ \times T/+$ (Johnson, 1974) show no evidence of the expected T^{hp}/T lethal. Further data on this cross and its reciprocal are presented below.

2. MATERIAL AND METHODS

Mice of the genotype t^{h2}/t^{h2} were obtained from the M.R.C. Radiobiology Unit, Harwell, Didcot, Berks., by courtesy of Dr M. F. Lyon. Mitotic preparations of the livers of 15-day-old embryos were made by a modification of the method of Evans, Breckon & Ford (1964).

3. RESULTS

Previous data (Johnson, 1974) from crosses of $T^{hp}/+ \times T^{hp}/+$ suggest strongly that the hairpin-tail gene inherited via the egg behaves differently from that contributed by the sperm. For formal proof, however, it is necessary to label the chromosome contributed by each parent with a marker. This could be done using genes situated close to T (such as H-2, tufted) in coupling. A quicker and neater solution, without the need to allow for recombination, is presented by the interaction of T^{hp} with t^{h2} to produce a tailless animal. If one parent is of the genotype T^{hp}/t^{h2} and the other $T^{hp}/+$ we can predict the offspring as follows:

			Offspring							
	Father	Mother	$+/t^{h2}$	:	$T^{hp}/+$	:	T^{hp}/t^{h2}	:	T^{hp}/T^{hp} (lethal)
(1)	T^{hp}/t^{h2}	$T^{hp}/+$	1	:	1	:	1	:	1
(2)	$T^{hp}/+$	T^{hp}/t^{h2}	1	:	1	:	1	:	1

If mice carrying T^{hp} derived from an egg are lethal then we shall expect no tailless T^{hp}/t^{h2} survivors at birth in cross 1, the survivors consisting of $1 + /t^{h2}$ (normal tail): $1\ T^{hp}/+^{1}$ (short tail). Similarly in cross 2 we should get $1 + /t^{h2} : 1\ T^{hp}/t^{h2}$, the T^{hp} allele in all cases originating in the sperm. In fact, just such results are obtained (Table 1). The small numbers of mice in Table 1 are the result of three factors. First, the cross $T^{hp}/+ \times t^{h2}/t^{h2}$ is relatively infertile; four matings produced only 69 offspring in 10 months. Secondly, of the 20 T^{hp}/t^{h2} mice born only two, one of each sex, reached maturity. Thirdly, the litter size in the cross $T^{hp}/t^{h2} \times T^{hp}/+$ is small, due to the 50 % embryonic loss *in utero*.

The observed phenotypic differences between $T^{hp}/+^{1}$ and $T^{hp}/+^{2}$ (Johnson, 1974) and the lethality of the latter seem to be due to the fact that the T^{hp} 'gene' (possibly a small deletion or other chromosomal reorganisation) is active in the haploid egg but inactive or ineffectual in the haploid sperm. The most common derangements of the mammalian egg concern the formation of the second polar body (Beatty, 1970). If T^{hp} is a chromosomal upset it is possible that the second meiotic division of the T^{hp} egg could be deranged. First, the second polar body might be suppressed and the resulting diploid egg fertilized by a + sperm to give

$T^{hp}/T^{hp}/+$, or by a T^{hp} sperm to give $T^{hp}/T^{hp}/T^{hp}$ triploid zygotes. Secondly, the sperm might take no part in fertilization, other than the initiation of development. In this case $T^{hp}/+^2$ would be a diploid zygoid T^{hp}/T^{hp} in constitution, and, as it contains two X chromosomes derived from the egg and second polar body respectively, always female.

Table 1. *Results of reciprocal crosses between mice of genotypes* $\mathrm{T^{hp}}/t^{\mathrm{h2}}$ *and* $\mathrm{T^{hp}}/+$

			Offspring				
	Father	Mother	$+/t^{h2}$ (normal)	$T^{hp}/+$ (short tail)	T^{hp}/t^{h2} (tail-less)	Total	Litter size
(1)	T^{hp}/t^{h2}	$T^{hp}/+$	10	10	0	20	2·8
(2)	$T^{hp}/+$	T^{hp}/t^{h2}	11	0	12	23	2·5

Table 2. *Sex of sectioned embryos from the hairpin-tail stock*

+/+		$T^{hp}/+^1$		$T^{hp}/+^2$	
♂	♀	♂	♀	♂	♀
1	3	3	0	2	3

Table 3. *Classification of embryos in reciprocal crosses between* $\mathrm{T^{hp}}/+$ *and* $\mathrm{T}/+$ *mice*

		Offspring					
Father	Mother	+/+	T/+	$T^{hp}/+$	Solid moles	Unclassifiable	Total
$T/+$	$T^{hp}/+$	21	27	13*	32	2	95
$T^{hp}/+$	$T/+$	21	36		20	1	78
$T/+$	+/+	22	19		3	0	44

* These can be distinguished from $T/+$ because they are of the $T^{hp}/+^2$ phenotype (see text). Mice were sacrificed on day 14 of pregnancy.

As a test of these hypotheses mitotic chromosome preparations of the livers of three $T^{hp}/+^1$, four $T^{hp}/+^2$ and two +/+ 15-day-old embryos were made. These were of sufficient quality to rule out any possibility of $T^{hp}/+^2$ being triploid and so exclude the first hypothesis, but not good enough to sex confidently. As an alternative the sex of 12 sectioned embryos was determined (Table 2). The $T^{hp}/+^2$ genotype contained both males and females, so invalidating the second hypothesis.

Because of the unusual effects of T^{hp} seen in reciprocal crosses it was decided that the search for the T/T^{hp} phenotype must be made in crosses between $T/+ \times T^{hp}/+$ reciprocally, as the T/T^{hp} compound might vary in phenotype as does $T^{hp}/+$. Crosses using a $T^{hp}/+$ mother allow the genotypes $T/+$ and $T^{hp}/+$ to be distinguished (Table 3). The expected ratio in offspring from this cross is 1 +/+ :

https://doi.org/10.1017/S0016672300015226 Published online by Cambridge University Press

1 $T/+$: 1 $T^{hp}/+^2$: 1 T^{hp}/T. In fact, no T^{hp}/T (presumed tailless) were seen, and the $T^{hp}/+^2$ class was deficient in numbers. The low viability of $T^{hp}/+^2$ embryos has already been noted. However, the number of solid moles is large, and even if some of these are assumed to be the missing $T^{hp}/+^2$ a considerable number remain unaccounted for. It is presumed that these represent the missing T/T^{hp} which may be supposed to die after implantation. Recognisable presumed T/T^{hp} embryos are also absent in the reciprocal cross with a $T/+$ mother (Table 3). Again there is an excess of solid moles presumably representing the T/T^{hp} genotype.

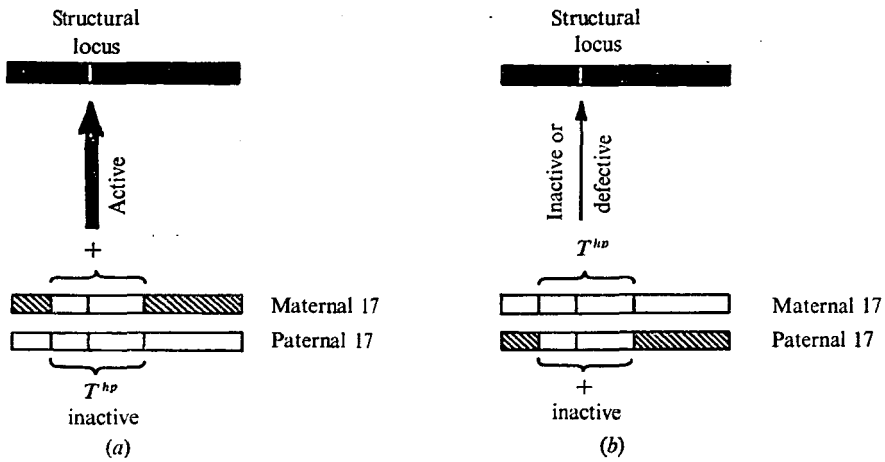

Fig. 1. Proposed mode of action of T^{hp} explaining the different phenotypes of the two heterozygotes. It is suggested that T^{hp} is a small deletion removing part of the brachyury (T) locus and also damaging the activator locus for a structur algene located elsewhere in the genome. (*a*) Represents the $T^{hp}/+^1$ heterozygote. By analogy with the work of Castro-Sierra & Ohno (1968) on the control of alcohol dehydrogenase in quail-chick hybrids it is supposed that the (normal) maternally derived activator locus alone is operative. In (*b*), the $T^{hp}/+^2$ heterozygote (which dies before birth), the maternal activator locus is presumed defective, resulting in the non-operation of the structural locus, which is necessary for survival. The defect in the T locus is conventionally expressed in both heterozygotes, irrespective of the origin of the chromosome. Normal chromosome 17 cross hatched, T^{hp} chromosome 17 white.

4. DISCUSSION

The only tenable explanation of the data on the segregation of T^{hp} is that the viability and phenotype of the heterozygote depend on whether the T^{hp} gene was transmitted via the sperm or via the egg. The sperm need not be considered further. In crosses of $T^{hp}/+♂ \times +/+♀$ (Johnson, 1974) a 1:1 ratio of viable offspring is produced in embryos old enough to classify accurately. This presupposes an adequate number of + and T^{hp} sperm produced in equal numbers. In addition no abnormality of sperm or testis could be found in conventional histological or electron microscope preparations (Johnson, unpublished).

The hypothesis that a chromosomal derangement could result in the $T^{hp}/+^2$

'heterozygote' being a diploid zygoid or a triploid entity is attractive, as such embryos would contain more than one dose of T^{hp}, which could account for the more extreme $T^{hp}/+^2$ phenotype. However, the two commonest chromosomal upsets in the egg (Beatty, 1970) must be rejected, as $T^{hp}/+^2$ are neither triploid nor exclusively female. Trisomy of all or part of chromosome 17 cannot be ruled out. It would, however, have to be limited to the egg.

The importance of the ovarian genotype in this situation must not be overlooked. Braden (1957) made reciprocal crosses between different strains of mice, and found that the strain used as female parent usually determined the incidence of first and second polar body suppression, cleavage of the egg at meiosis and the occurrence of uninucleate eggs and those with subnuclei. As the T^{hp} egg can only develop in the T^{hp} ovary it is impossible to asses the effect of ovarian genotype. However, the cells destined to become + eggs in these ovaries (which must be considered identical to those destined to become T^{hp} eggs until + and T^{hp} segregate at the first meiotic division, about 10 h before ovulation in the mouse) are apparently unaffected by their T^{hp} environment.

The enumeration of possible differences between the + and T^{hp} gene is hampered by our almost total ignorance of early gene action in the mammalian egg. One obvious possibility is that during embryogenesis the genes on maternally and paternally derived chromosomes are not activated simultaneously, but that transcription of the paternal genome lags by some unknown amount. This would expose an embryo containing a maternally derived T^{hp} gene to the effects of the gene (whatever they may be) for a period before the situation was corrected or ameliorated by a paternal +. Unfortunately there is no evidence to support this hypothesis. Our knowledge of the time of activation of the embryonic genome in mammals is strictly limited. Epstein (1972) was able to show, by the use of *XX versus XO* ♀♀, that embryonic HGPRT was first seen on day 3 of embryonic life, i.e. morula/blastula stage. Lactic dehydrogenase (LDH) can first be detected in 9-day embryos (Epstein, Weston, Whitton & Russell, 1972). Most pertinent however is the work of Chapman, Whitten & Ruddle (1971) on glucose phosphate isomerase-1 (Gpi-1). They used mouse strains carrying different electrophoretic variants of this enzyme and were able to show that the paternal type was present from day 4 onwards. This technique is unfortunately unable to estimate the time of action of the maternally derived component, as this is the same as that of the egg cytoplasm, whose activity was detectable throughout.

Other investigations pertinent to the problem were made by Ohno and his coworkers (Castro-Sierra & Ohno, 1968; Ohno, Stenius, Christian & Harris, 1968; Ohno, Christian, Castro-Sierra & Muramato, 1969). They worked on chick/quail hybrids differing in alcohol dehydrogenase (ADH) isoenzyme type, and found that, up to hatching, the allele expressed was always the maternal one, irrespective of the way in which the cross was made. Ohno postulated that a control centre for ADH loci in both chromosomes must reside in the maternal set, and that mutational events since the separation of quail and chick from a common ancestor must have led to mutual incompatibility, the control centre of one species being unable

to activate the ADH gene of the other. Chick/chick and quail/quail crosses showed that both ADH isoalleles were activated simultaneously.

Could not a similar situation exist in T^{hp}? If we postulate that the T^{hp} gene contains an activation centre for some unknown but necessary process, and that in mutant form this is partly or totally defective, then a situation arises which is compatible with the results obtained (Fig. 1). It will be noted that the type 1 heterozygote ($T^{hp}/+^1$) is normal with respect to the activation of the second locus: this does not mean that it is normal with respect to tail length. We know that T^{hp} maps with T on chromosome 17 and affects tail length. It is possible that the hypothetical control locus affected by the T^{hp} mutation (but not, apparently by other known mutations in the T region) lies immediately adjacent to T, and that T^{hp} is in fact a small deletion removing parts of both loci. A parallel situation has recently been described with respect to the albino locus (*c*) where lethal alleles affect both *c*, giving an albino phenotype, and adjacent regions concerned with enzyme synthesis (Thorndike, Trigg, Stockhert, Gluecksohn-Waelsch & Cori, 1973; Trigg & Gluecksohn-Waelsch, 1973). In this case the defect is in the structure of biological membranes upon which membrane bound enzymes reside.

The problem of what exactly T^{hp} does, and how, should be viewed in the context of the T region of chromosome 17. The T region embraces many t alleles which show unorthodox transmission in the male: many of these suppress crossing over in the vicinity of T, but when such crossovers do occur they are a source of new t alleles of different properties. Unexplained maternal effects are found in $T/t^{1\,"Br"}$ *Fu* and *Ki* (fused, *Fu* and kinky, *Ki* are located near the T region of chromosome 17; Gluecksohn-Waelsch & Erickson 1970) and various combinations of T and t^{y} have profound developmental effects on the notochord and mesoderm of the developing embryo. Many t alleles exhibit abnormal segregation ratios when transmitted via the sperm: T^{hp} has added a new facet to this fascinating locus by acting in the egg.

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