Genet. Res., Camb. (1984), **44**, *pp.* 343–346
Printed in Great Britain

SHORT PAPER

Genetic differences between two substrains of the inbred 101 mouse strain

By JOHN D. WEST, JOSEPHINE PETERS and MARY F. LYON

MRC Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD, England

(*Received 29 June 1984*)

SUMMARY

Two substrains of the inbred mouse strain 101, maintained at Harwell and at Neuherberg, and designated 101/H and 101/El, differed at five of the eight genetic loci tested and it seems very likely that one of these substrains has been genetically contaminated. The available evidence suggests that 101/El is probably the contaminated substrain.

INTRODUCTION

The inbred mouse strain 101 was originally developed by Dunn who also developed the well-known strain 129 from the same general source (see for example Festing, 1979, p. 138). Strain 101 is now used largely in mutagenesis experiments in which the treated animals are (101 ♀ × C3H ♂) or (C3H ♀ × 101 ♂)F_1 hybrids. Dunn passed the strain to W. L. Russell at Oak Ridge in 1947–8. He in turn passed it to Carter in Edinburgh in 1953 (and thence to Harwell in 1954), and to Ehling at Neuherberg in 1961. Three substrains of the strain were thus formed, designated 101/Rl, 101/H and 101/El.

During the course of a screen for genetically inherited cataracts it was discovered that mice of the 101/H strain, maintained at Harwell, had cataracts (West & Fisher, in preparation). The 101/El substrain had been used as parents in extensive studies of cataracts induced in offspring by mutagens and the control animals were uniformly negative (Kratochvilova & Ehling, 1979; Ehling *et al.* 1982; Favor, 1983). It thus seemed most unlikely that cataracts were present in the 101/El substrain maintained at the Institut Für Genetik at Neuherberg.

In order to test this, and with the kind co-operation of Dr U. Ehling and Dr A. Neuhauser-Klaus, we imported the 101/El strain from Neuherberg for comparison with our own 101/H strain. We found differences not only in cataract but also at various other genetic loci.

MATERIALS AND METHODS

Blood samples from mice of the Neuherberg 101/El strain and two Harwell strains, 101/H and C3H/HeH, were typed for five loci (*Car-2*, *Hba*, *Gpi-1s*, *Hbb* and *Pgm-1*) by isoelectric focusing of carbonic anhydrase-2, and haemoglobin alpha-chain (Whitney *et al.* 1979) and by cellulose acetate electrophoresis of glucose phosphate isomerase, haemoglobin beta-chain and phosphoglucomutase-1 respectively (Eicher & Washburn, 1978; Whitney, 1978; Loutit, Peters & Marshall, 1981; West & Green, 1983).

In addition, the presence of cataracts (*lop-2*) was detected using a slit lamp to examine the lens after the pupil had been dilated with a drop of 1 % (w/v) atropine sulphate (Evans Medical Ltd.). Mice homozygous for retinal degeneration (*rd*) were identified by the

https://doi.org/10.1017/S0016672300026562 Published online by Cambridge University Press

absence of the outer nuclear layer of the neural retina in histological sections of the eye, stained with haematoxylin and eosin. Mice carrying white-bellied agouti (A^w) were discriminated from those homozygous for agouti (A) by visual inspection of the coat.

The eight original sibling 101/El mice that were imported from Neuherberg were typed for *a*, *Car-2*, *Gpi-1s*, *Hba*, *Hbb* and *Pgm-1* and their progeny were used to type the strain for *rd* and *lop-2*.

RESULTS

The results (Table 1) show that 101/El and 101/H share the same alleles at only three of the eight loci tested. The same *Hbb* allele (Hbb^d) is present in 101/H, 101/El and C3H/HeH but the alleles of *Pgm-1* and *rd* (both on chromosome 5) differ between C3H/HeH and the two 101 strains. For the remaining five loci tested the 101/El strain shares alleles with C3H/HeH but not 101/H. All eight 101/El mice that were imported from Neuherberg were homozygous for the same allele at *Car-2*, *Gpi-1s*, *Hba*, *Hbb* and *Pgm-1*.

In addition a subline of 101/H separated from the Harwell colony in 1971 and maintained by Dr E. P. Evans in the Sir William Dunn School of Pathology, Oxford also carried A^w and *lop-2* (West & Fisher, in preparation).

Table 1. *Genetic differences between 101/El, 101/H and C3H/HeH strains of mice*

		Alleles present		
Locus	Chromosome	101/ H	101/El	C3H/HeH
Hbb	7	d	d	d
Pgm-1	5	a	a	b
rd	5	+	+	rd
a	2	A^w	A	A
Car-2	3	a	b	b
Gpi-1s	7	a	b	b
Hba	11	a	c	c
*lop-2**	?	lop-2	+	+

* *lop-2* is the provisional gene symbol used for the cataract found in 101/H mice (West & Fisher, in preparation).

DISCUSSION

The genetic differences between 101/H and 101/El could be explained either by subline divergence or if they are unrelated inbred strains, one of which has been incorrectly named. Bailey (1978) and Morse (1978) considered three sources of subline divergence in inbred strains: (1) contamination from outcrossing, (2) incomplete inbreeding and (3) mutation. From the magnitude of the genetic differences shown in Table 1 only the first of these three possibilities seems likely.

As mentioned earlier the 101 strain at Oak Ridge, Neuherberg and Harwell is principally used to produce (101 ♀ × C3H ♂)F_1 or (C3H ♀ × 101 ♂)F_1 hybrids for use in mutation experiments. Outside these three centres 101 is a relatively little used strain and its genotype is not as well documented as those of the more common inbred strains. All three substrains (101/Rl, 101/H and 101/El) are listed as A^w (Inbred Strains of Mice No. 13, Companion to Mouse News Letter, No. 69, July 1983). The discrepancy between this listing for 101/El and the agouti (A) phenotype of the 101/El mice that we examined suggests that 101/El is not the 'true' 101 strain. Moreover Russell *et al.* (1976) list 101/Rl as A^w, Hba^a and Hbb^d which agrees with our observations for 101/H whereas 101/El was A, Hba^c and Hbb^d (Table 1). Roderick, Staats & Womack (1981) list 101 as

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Gpi-1s^{a} which agrees with our results for 101/H. However, this does not help us to decide which subline is the 'true' 101 strain since the listed information was probably based on 101/H (see Staats, 1980).

The listing of 101/Rl as A^{w} and *Hba*^{a} is sufficient to suggest that 101/El is less likely to be a 'true' 101 strain than 101/H. 101/El could either be another strain that has been incorrectly identified or a recombinant inbred strain produced by genetic contamination of 101 by another strain (possibly C3H/He).

The results for 101/El (Table 1) were compared with the alleles for *Car-2*, *Gpi-1s*, *Hba*, *Hbb*, *Pgm-1* and *rd* listed by Roderick *et al.* (1981) for 80 inbred strains of mice. Comparison of alleles at these six loci and the coat colours (documented by Festing, 1979) showed that 101/El differed from 78 of the 80 strains considered. The remaining two strains (CHI/– and NZO/–) were both listed as agouti but there was insufficient information on the other loci considered. None of the relevant genes were listed for NZO and only rd^{+} was listed for CHI.

It seems unlikely that 101/El is actually one of the 80 inbred strains, listed by Roderick *et al.* (1981), that has been incorrectly labelled. Genetic contamination of the 101/El strain, therefore, appears to be the most probable explanation of the differences between 101/El and 101/H (Table 1) and the discrepancy between the agouti phenotype of 101/El and the listing of all three substrains as white-bellied agouti.

Other examples of probable genetic contamination of inbred strains of mice include the origin of the A2G strain from strain A (see Festing, 1979, p. 151) and the genetic differences between C3H/He and C3H/Bi (McLaren & Tait, 1969), between CBA/Ca and CBA/J (Roderick, 1978), between C57BL/Ks and other C57BL strains (see Bailey, 1978; Morse, 1978) and between commercially supplied BALB/c mice (Kahan *et al.* 1982).

The question now arises whether the magnitude of the difference between 101/H and 101/El warrants their designation as two distinct strains or two substrains of the same strain. The strains have been shown to differ at 5 out of 8 loci tested. Although this is only a small sample of loci, it does suggest that the difference between the strains is extensive. According to Roderick *et al.* (1981), strains CBA/Ca and CBA/J differ at 11 out of 49, and strains C57BL/KsJ and C57BL/6 at 7 out of 54, loci tested. Thus, in these two cases the discrepancies are less extensive than in the 101 strains. Furthermore, strains A/– and A2G, which are regarded as two separate strains, differ at only 3 out of 36 tested loci. The differences between 101/H and 101/El seem more on a par with those which might be expected between two conventionally derived recombinant inbred strains, rather than two substrains of the same strain. Thus it might be advisable to designate them as two separate strains. Further studies on other loci in these strains, and in 101/Rl would be very valuable.

Whatever the origin of the genetic differences between 101/H and 101/El these differences have disturbing implications for the interpretation of experiments, including many mutation studies, where different substrains of 101 have been used. This is particularly so in view of the report that the 101/HY subline (derived from the Harwell 101/H colony in 1969) may be unduly sensitive to the mutagenic effect of thio-TEPA (Surkova & Malashenko, 1977).

We are very grateful to Drs A. Neuhäuser-Klaus and U. Ehling for sending us 101/El mice and to Dr E. P. Evans for allowing us to examine 101/H mice from his colony. We also thank Mr S. Ball and Mr G. Fisher for technical assistance with the electrophoresis and Mr D. Beaney, of our histology department, for preparing sections of eyes.

https://doi.org/10.1017/S0016672300026562 Published online by Cambridge University Press

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