

SHORT PAPER

Chromosome differences between substrains of the inbred 101 mouse strain

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SUMMARY

A centromeric heterochromatin (Hc) comparison of two substrains of the mouse inbred strain 101 with another two, revealed differences in at least two and possibly in up to six chromosome pairs. The differences could have arisen by the inclusion of chromosomes from the inbred strain C3H/He.

1. INTRODUCTION

Two substrains of the inbred mouse strain 101, maintained at Harwell and Neuherberg, were found by West, Peters & Lyon (1984) to differ at five out of the eight genetic loci tested and they considered it a possibility that the Neuherberg substrain had been contaminated by the inbred C3H/He strain. During several years study of the G and C-band patterns of the Harwell-derived (101♀ × C3H♂) or (C3H♀ × 101♂) F1 hybrids, we had observed a constant heterozygosity in certain homologous pairs of chromosomes and confirmed that some differences existed between the karyotypes of these 101 and C3H/He substrains. More recently, in studies involving the same F1 crosses from Neuherberg, we failed to observe this heterozygosity which suggested that no such differences existed. In order to resolve this enigma and with the kind cooperation of Dr Mary F. Lyon's Group at Harwell, we decided to compare the G-banded karyotypes of the 101 and C3H/He strains from Harwell and from Neuherberg and also to compare them to the karyotypes of two 101 substrains from Oak Ridge.

The comparisons confirmed the genetic conclusions (West *et al.* 1984) that the Neuherberg substrain had been contaminated, probably by the C3H/He strain. They also showed that the two Oak Ridge substrains differed from each other but were 'true' to their historical origin.

2. MATERIALS AND METHODS

The history and designations of three of the four 101 substrains have been described (West *et al.* 1984). In brief, 101/Rl (for W. L. Russell at Oak Ridge) was first sent to Edinburgh in 1953 and from there to Harwell in 1954 to become 101/H. The offshoot 101/HOxe was formed in 1971 by 101/H mice sent to Oxford. In 1961, 101/Rl was also sent from Oak Ridge to Neuherberg to become 101/El (for Ehling). The fourth substrain,

101/Sl (for Selby) was derived from 101/El by importation to Oak Ridge from Neuherberg in 1976.

Karyotypes were produced for all four 101 substrains and also for two C3H/He substrains, C3H/HeHOxe (an offshoot of the Harwell C3H/He substrain sent to Oxford in 1971) and C3H/HeEl from Neuherberg. These were prepared from air-dried preparations made from direct bone-marrow (Ford, 1966) without using a mitotic arrestant and with aqueous 0.56% (w/v) Potassium chloride as the hypotonic treatment; mitotic cells were G-banded by a modification of a combined ASG/trypsin method (Gallimore & Richardson, 1973). Suitably spread and G-banded cells were photographed under a $\times 100$ oil immersion lens. The chromosomes were cut out from the photographic prints obtained and compared by visual inspection. Up to ten prints were analysed for each substrain.

The G-bands were identified according to the standard idiogram (Nesbitt & Francke, 1973) and the size of the centromeric heterochromatin regions (which are synonymous to the A1 G-bands) compared by part reference to published information (for example, see Davisson, 1981). For purposes of nomenclature, this region has been designated by the symbol Hc. It is known to vary in size in up to five chromosome pairs in some inbred strains if compared to the accepted 'normal' which is the size of Hc regions in the C57BL/6J strain. The variations in size, followed by chromosome number, are expressed as Hc^s for small, Hc^l for large and Hcⁿ for normal, if of the same size as those in C57BL/6J. Unfortunately, the published information does not encompass all the visible Hc size variations between inbred strains since it is based on comparisons of relatively gross differences and their departures from the standard karyotype of the one inbred strain. It is our experience that comparisons made between the chromosomes of inbred strains can reveal many more, some of them albeit small, differences than are suggested by the existing list of centromeric heterochromatin variants (see for example, Davisson, 1981) and which cannot be accommodated by the existing nomenclature. In our comparisons, we have made use of these differences and describe Hc sizes as being large or small relative to the chromosome pairs within the 101 and C3H/He substrains studied. Although we studied G-banded chromosomes and did not, in the strictest sense, observe Hc regions but the synonymous A1 G-band regions, it is more convenient to refer to them as Hc since, for these, strain variants have been recorded but A1 band variants are not mentioned in the standard idiogram.

3. RESULTS

Apart from the differences in the sizes of the A1 bands (hereafter referred to as Hc), no other G-band pattern differences were observed in the comparison of karyotypes from the four 101 and the two C3H/He substrains. Within the four 101 substrains, Hc differences were observed in chromosome pairs 1, 2, 7, 8, 13 and 17 but since some of them were subtle and sometimes subject to the possibility of other variation resulting, for example, from technical differences between slides, overall comparisons between all the substrains were restricted to the most extreme differences which were observed in pairs 8 and 13. The comparisons show (Plate 1) that both the 101/HOxe and 101/Rl substrains have small Hc8 but large Hc13 whereas the 101/El and 101/Sl have the reverse, large Hc8 and small Hc13. In size, these are more akin to the Hc regions of these chromosome pairs from the two C3H/He substrains.

Since, at least with respect to chromosome pairs 8 and 13, 101/HOxe appeared to be the same as 101/Rl, and 101/El the same as 101/Sl, a more detailed and controlled comparison of Hc sizes was made between all the chromosomes from karyotypes of the 101/HOxe, 101/El and C3H/HeHOxe substrains. No consistent differences were observed in ten chromosome pairs (pairs 3, 4, 6, 9, 10, 11, 12, 14, 16 and 19) nor in the Y chromosome. In three pairs (Pairs 5, 15 and 18) and in the X chromosome, 101/HOxe was considered equivalent to 101/El, both differing from C3H/HeHOxe. In the

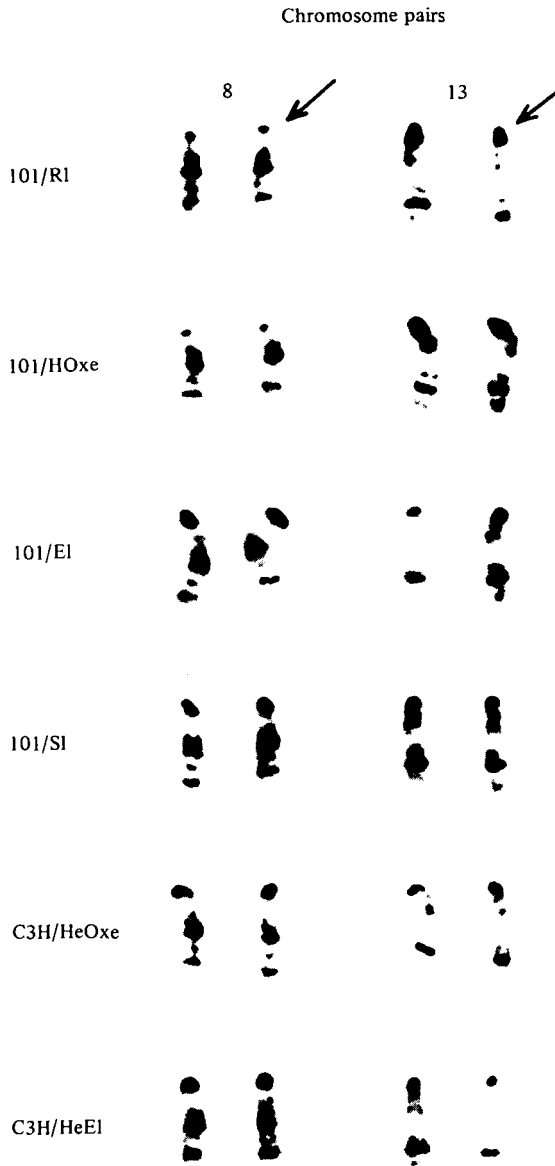


PLATE 1

A comparison between Hc (arrowed) sizes in chromosome pairs 8 and 13 from the four 101 and the two C3H/He substrains.

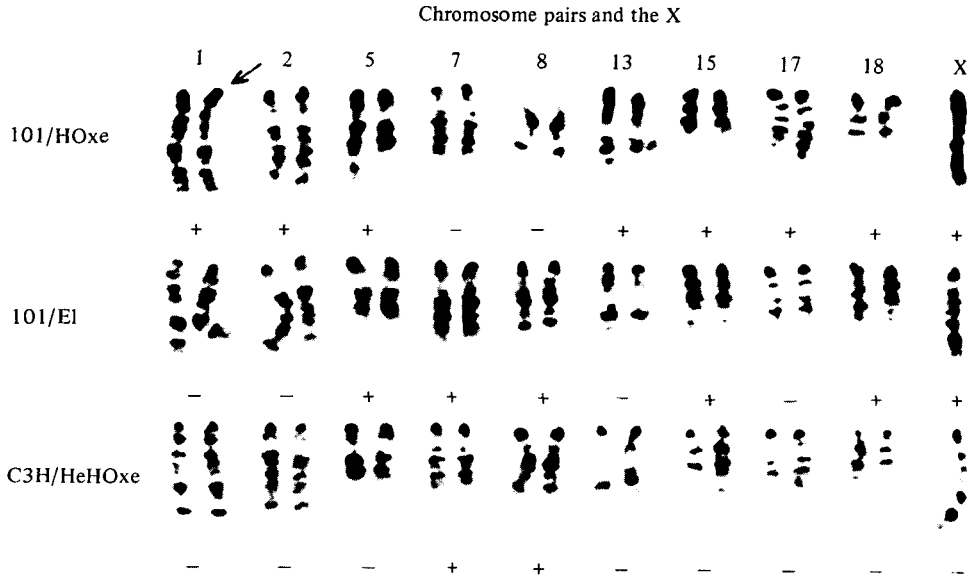


PLATE 2

A comparison between Hc (arrowed) sizes in chromosome pairs 1, 2, 5, 7, 8, 13, 15, 17 and 18 and in the X chromosome from two of the 101 and one of the C3H/He substrains. Hc sizes considered larger are indicated by a + sign placed beneath the pairs and if considered smaller, by a - sign. If the two signs correspond, the pairs are considered equivalent.

remaining six pairs (pairs 1, 2, 7, 8, 13 and 17), 101/El was considered more equivalent to C3H/HeHOxe than to 101/HOxe (Plate 2).

4. DISCUSSION

From the genetic differences found between 101/H and 101/El, West *et al.* (1984) concluded that the most likely source of subline divergence stemmed from the contamination of 101/El by another strain which was possibly C3H/He. Our observations on the karyotypes confirm this conclusion and also suggest that the substrain carries at least two chromosome pairs (8 and 13) and possibly a further four (1, 2, 7 and 17), which could be of C3H/He origin.

The observations also support the contentions of West *et al.* (1984) regarding the history of 101/H. Since the karyotypes of 101/H and 101/Rl appear identical within the limitations of our study, they must represent the 'true' 101 strain as originally developed by Dunn and sent to Oak Ridge in 1947–8. Further, the observations of the same karyotype markers in 101/El and in 101/SI show, as expected from the relatively recent importation of the latter, that they are chromosomally related and carry the same introduced chromosomes.

It is of interest to compare the genetic findings of West *et al.* (1984) with our findings. They tested seven mapped loci, of which one was uninformative since the same allele was present in all three substrains. Of the other six, two, on chromosome 5, were found to be the same in 101/H and in 101/El but different in C3H/HeH. The remaining four loci, on chromosomes 2, 3, 7 and 11, were different in the two 101 substrains but the same in 101/El as in C3H/HeH. In our chromosome study, and assuming from the evidence obtained from the karyotypes that the Oxford substrains are the same as the Harwell ones, we concluded that chromosome 5 was equivalent in the two 101 substrains but different in C3H/He. Chromosomes 2 and 7 were equivalent in 101/El and in C3H/He but different in 101/H and chromosomes 3 and 11 were not informative since they had equivalent sized Hc regions in all three substrains. The genetic and chromosomal findings are therefore compatible for the three informative chromosomes and if suitable variants were available, it would be of interest to compare loci on the other four chromosome pairs (1, 8, 13 and 17) which we considered to be more equivalent in 101/El to those of C3H/He rather than to those of 101/HOxe.

The C3H/He but not the 101 strain is included in the list of centromeric heterochromatin variants in inbred strains (see Davisson, 1981). It is possible, however, that the latter may have similar variants to the listed 129 strain which was derived from the same source (see for example Festing, 1979). In the comparison to the normal standard of the Hc region sizes in the C57BL/6J strain, the C3H/HeJ is listed as having Hc1^s, Hc14^s and Hc18^s and the 129 as having Hc8^s and Hc14^s. We observed small Hc in chromosomes 1 and 18 (Plate 2) and in chromosome 14 (not illustrated) from the C3H/HeHOxe substrain. In strain 101/HOxe, we observed small Hc in both chromosomes 8 (Plate 2) and 14 (not illustrated) consistent with it having a common origin with strain 129. Strain 101/El, however, had small Hc on chromosome 14, but large or normal sized Hc on chromosome 8 (Plate 2). This provides further evidence that 101/El, rather than 101/H, is the contaminated strain.

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