CYTOLOGICAL DETECTION OF THE c^{25H} DELETION INVOLVING THE ALBINO (c) LOCUS ON CHROMOSOME 7 IN THE MOUSE[1]

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ABSTRACT

A deletion of the albino (c) locus on mouse chromosome 7 has been demonstrated using Q- and G-banding methods in a mouse heterozygous for the radiation-induced lethal albino allele, c^{25H}. The deletion, which is thought to be 1–6 cM long, represents about 7.6% of the length of the metaphase chromosome.

COMPLEMENTATION studies of a series of radiation-induced mutations at the albino (c) locus with multiple biochemical and ultrastructural effects led to the distinction of four different complementation groups among six lethal alleles, supporting the interpretation that these represent overlapping deletions of various sizes (GLUECKSOHN-WAELSCH *et al.* 1974). Homozygotes for each of the six alleles were lethal, four of them dying perinatally and two during early embryogenesis. No complementation was observed in combinations of any of the other five alleles with c^{25H}, which is thought to be the biggest deletion. The complementation studies included determinations of activity of the enzymes glucose-6-phosphatase, tyrosine aminotransferase and serine dehydratase, serum protein measurements, and examination of subcellular ultrastructure, viability, growth, fertility and morphogenesis (GLUECKSOHN-WAELSCH *et al.* 1974). The c^{25H} mutation was shown to be deleted at the structural locus for the enzyme mitochondrial malic dehydrogenase *mod-2* (ERICKSON, EICHER and GLUECKSOHN-WAELSCH 1974).

Previous genetic studies attempting to determine the limits of possible chromosomal deletions in the radiation-induced c-alleles made use of two outside markers: taupe (proximally, 2% recombination with albino), and shaker-1 (distally, 4% recombination with albino). Both of these loci were found to be intact in c^{14CoS}, c^{112K}, c^{65K}, and c^{3H} (GLUECKSOHN-WAELSCH and CORI 1970).

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Although c^{25H} was not available at the time of these studies, it is assumed that this deletion, too, does not extend as far as the taupe locus in one direction or the shaker-1 locus in the other.

The introduction of chromosome banding techniques greatly increased the accuracy of detection of cytological abnormalities in various mammalian species. However, few attempts have yet been made to apply banding techniques to the recognition of presumptive deletions, and the first of these was unsuccessful (DEV *et al.* 1971). Linkage group 1, which carries the *c* locus, was assigned to chromosome 7 in the mouse (KOURI *et al.* 1971; MILLER *et al.* 1971b; Committee 1972). We report here the presence of a visible deletion in chromosome 7 in mice heterozygous for c^{25H}.

MATERIALS AND METHODS

A male mouse heterozygous for c^{25H} (c^{25H}/c^{ch}) was mated to two AKR females. The pregnant females, which were sacrificed on day 14 or 15, produced nine albino (c^{25H}/c) and three non-albino (c^{ch}/c) fetuses. The albino embryos were minced and pooled, and primary cultures were set up as described previously (MILLER *et al.* 1971a). Control cultures from non-albino littermates were set up in the same way. After two days in culture, mitotic cells were harvested by mild trypsinization to remove the loosely attached cells. The freed cells were transferred to a centrifuge tube containing a drop of Colcemid (10μg/ml) and spun at 600 rpm. The medium was replaced with 0.07 M KCl, left at room temperature for 15 minutes and then replaced, after centrifuging, with methanol : glacial acetic acid (3:1). After three changes of fixative, the suspended cells were dropped onto chilled, wet slides, air dried, and stored at 4°. Within 6 days some of the slides were stained with quinacrine mustard (.05 μg/ml) for eight minutes, rinsed with water and tris-maleate buffer at pH 5.6, and mounted in the same buffer. The slides were examined either by transmitted or incident UV light (HBO 200 watt mercury bulb illumination), BG 12 exciter filter and K530 barrier filter, photographed on H&W control film with 30–40 second exposures, developed in H&W developer and printed on Ilford paper. Other slides were stained for G-banding by the method of SUN, CHU and CHANG (1973) and photographic prints made. About 100 Q- and G-banded metaphase prints were made on the albino embryos, and 42 karyotypes were prepared. About 40 prints were made of the normal embryos, and 12 karyotypes were prepared.

The chromosomes 7 were measured on prints of 28 heterozygous and 10 normal metaphase cells using a ruler marked at 1/64-inch intervals. Each chromatid was measured twice and the four values were summed.

RESULTS

The control cultures showed normal karyotypes. The cultures of the c^{25H}/c albino embryos were a mixture of XX and XY cells which showed one consistent abnormality. Using quinacrine staining the more distal bright band normally present in the middle region of chromosome 7 was absent from one chromosome in both the male and female embryos. A less bright band was sometimes present closer to the distal end of the chromosome, probably representing the secondary band which is observed only in the most clearly banded chromosomes. Similar results were obtained using Giemsa-banding (Figures 1 and 2). Measurements showed that the deleted chromosome was 7.6 $\pm$ 1.5% shorter than its homolog. The difference between the longer and shorter chromosome 7 in these cells, without regard to identifying the deleted chromosome, was about

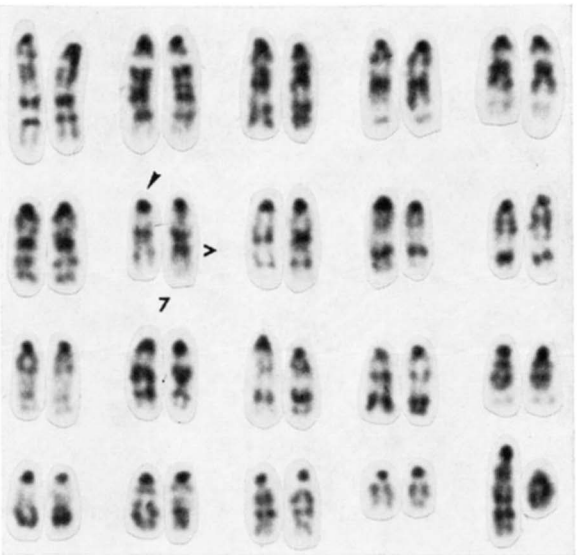

FIGURE 1.—Giemsa-banded karyotype of a mouse heterozygous for c^{25H} deletion. The arrow points to the deleted chromosome 7. The band which has been deleted from the normal chromosome 7 is indicated by a >.

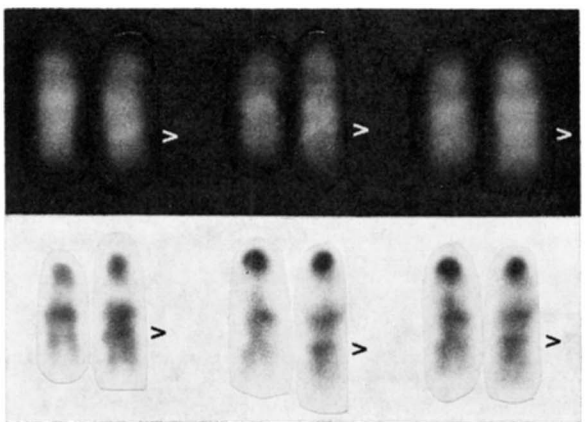

FIGURE 2.—Chromosomes 7 from a series of metaphases stained with quinacrine or trypsin-Giemsa from mice heterozygous for c^{25H}. A band is missing from the left chromosome of each pair. The band which has been deleted is indicated on the normal chromosome by a >.

8.8 ± 1.2%. In the control cells the maximum mean difference in length between the longer and the shorter chromosome 7 was 5.3 ± 1.0%.

DISCUSSION

Our results indicate that even a small deletion, as defined genetically, can be visible cytologically. To our knowledge this is the first time a deletion has been demonstrated in mitotic chromosomes in the mouse. The c^{25H} deletion is probably less than six centimorgans (cM) long, since that is the distance between the presumably intact outside markers *sh-1* and *tp*, but more than 1 cM long, because both *mod-2* and *c* loci are absent (Figure 3). A deletion of this size might have been suspected by length measurements alone, in the absence of a chromosome

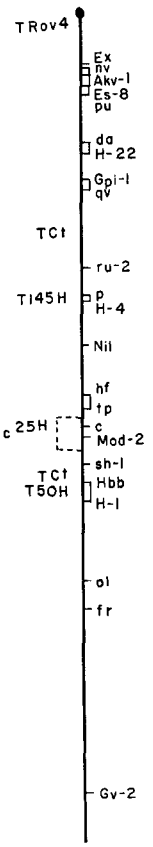

FIGURE 3.—Mouse Linkage Group I (chromosome 7). Symbols to the left of the line indicate the relative locations of translocations which have been mapped both genetically and cytologically. The approximate extent of the c^{25H} deletion is indicated by a dashed line. Symbols to the right of the line indicate the relative locations of genes; the full name of each gene may be found in Mouse News Letter. Where the order of loci is uncertain, the locations are connected by narrow vertical lines. The centromere is shown as a dark circle. The distance from *Ex* to *Gv-2* is about 80 cM; the distance from the centromere to *Ex* is uncertain. (Prepared in part from data compiled by DR. MARGARET C. GREEN.)

marker. In the littermate controls the difference in length between the longer and shorter normal chromosomes 7 was 5.3 ± 1.0%, reflecting random variations in the length of the homologs due to biological or technical factors. In the albino embryos the longer chromosome 7 in each cell (which was usually the normal 7 but sometimes the deleted 7) was 8.8 ± 1.2% longer than the shorter homolog, or about 3.5% greater than expected if there had been no deletion.

The ability to identify the deleted chromosome 7 on the basis of its banding pattern permits a more accurate determination of the size of the deletion. We assume that in the normal littermates the two chromosomes 7 are the same length and the observed difference in their length in any cell is the result of random factors. That is, if we could consistently distinguish between the maternally and paternally derived chromosome we would find no difference in the average length. In the heterozygous animals we *can* distinguish the two chromosomes 7 on the basis of their banding patterns. We again assume that any technical variation will affect both chromosomes to the same extent. However, instead of finding no difference in the average length we find a difference of 7.6 ± 1.5%. This is a direct estimate of the size of the deletion.

The total genetic length of the mouse genome has been estimated, from chiasma frequencies in male and females, to be 1200–1300 cM (G. JAGIELLO and P. E. POLANI, personal communication). Chromosome 7 makes up about 5.4% of the haploid autosome complement at metaphase (Committee, 1972), so that a deletion of 7.6% of this chromosome might be expected to be about 5 cM long. This is in the range of the length of the c^{25H} deletion based on the available genetic recombination data.

The c^{25H} deletion was detectable cytologically because a normally brightly fluorescent band was absent from one chromosome 7 in the c^{25H}/c heterozygous albino embryos. Such bright Q-bands appear to be very AT-rich since quinacrine fluorescence is enhanced in AT-rich regions of DNA in solution and quenched in GC-rich regions (WEISBLUM and DE HASETH 1972). Furthermore, the same regions preferentially bind antibodies specific for adenosine, producing, in general, a Q-banding pattern (DEV *et al.* 1972), whereas antibodies specific for cytosine produce the reverse R-banding pattern (SCHRECK *et al.* 1973). Several lines of evidence support the idea that the AT-rich bands contain a great deal of repetitious DNA. CRICK (1971) has suggested that repetitious DNA may play an important regulatory role, rather than specifying amino acid sequences in proteins. The broad range of abnormalities observed both prenatally and postnatally in homozygotes and double heterozygotes for lethal alleles at the albino locus may thus be the result of the deletion of both structural and regulatory genes. This agrees with conclusions reached also as a result of previous biochemical, developmental and complementation studies (ERICKSON *et al.* 1974).

Not all deletions can be expected to be identified cytologically with equal ease, even if they are the same length judged by genetic mapping. Not only is the banding pattern in the neighborhood of the deletion an important factor in detectability, but the correlation between cytologically observed length and map distance is not a simple linear function (BRIDGES 1935; LEFEVRE 1971). We are

currently trying to identify the presumptive deletion in each of the other mutants involving the albino locus referred to earlier.

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LITERATURE CITED

Bridges, C. B., 1935 Salivary chromosome maps. J. Heredity **26:** 60.

Committee on standardized genetic nomenclature for mice, 1972 Standard karyotype of the mouse, *Mus musculus*. J. Hered. **63:** 69–72.

Crick, F., 1971 General model for the chromosomes of higher organisms. Nature **234:** 25–27.

Dev, V. G., M. S. Grewal, D. A. Miller, R. E. Kouri, J. J. Hutton and O. J. Miller, 1971 The quinacrine fluorescent karyotype of *Mus musculus* and the demonstration of strain differences in secondary constrictions. Cytogenetics **10:** 436–451.

Dev, V. G., D. Warburton, O. J. Miller, D. A. Miller, B. F. Erlanger and S. M. Beiser, 1972 Consistent pattern of binding of anti-adenosine antibodies to human metaphase chromosomes. Exptl. Cell Res. **74:** 288–293.

Erickson, R. P., E. M. Eicher and S. Gluecksohn-Waelsch, 1974 Demonstration in the mouse of x-ray induced deletions for a known enzyme structural locus. Nature **248:** 416–418.

Erickson, R. P., P. Siekevitz, K. Jacobs and S. Gluecksohn-Waelsch, 1974 Chemical and immunological studies of liver microsomes from mouse mutants with ultrastructurally abnormal hepatic endoplasmic reticulum. Biochem. Genet. **12:** 81–95.

Gluecksohn-Waelsch, S. and C. F. Cori, 1970 Glucose-6-phosphatase deficiency: mechanisms of genetic control and biochemistry. Biochem. Genet. **4:** 195–201.

Gluecksohn-Waelsch, S., M. B. Schiffman, J. Thorndike and C. F. Cori, 1974 Complementation studies of lethal alleles in the mouse causing deficiencies of glucose-6-phosphatase, tyrosine aminotransferase and serine dehydratase. Proc. Natl. Acad. Sci. U.S. **71:** 825–829.

Kouri, R. E., D. A. Miller, O. J. Miller, V. G. Dev, M. S. Grewal and J. J. Hutton, 1971 Identification by quinacrine fluorescence of the chromosome carrying mouse linkage group I in the Cattanach translocation. Genetics **69:** 129–132.

Lefevre, G., Jr., 1971 Salivary chromosome bands and the frequency of crossing over in *Drosophila melanogaster*. Genetics **67:** 497–513.

Miller, O. J., D. A. Miller, R. E. Kouri, P. W. Allderdice, V. G. Dev, M. S. Grewal and J. J. Hutton, 1971a Identification of the mouse karyotype by quinacrine fluorescence, and tentative assignment of seven linkage groups. Proc. Natl. Acad. Sci. U.S. **68:** 1530–1533.

Miller, O. J., D. A. Miller, R. E. Kouri, V. G. Dev, M. S. Grewal and J. J. Hutton, 1971b Assignment of linkage groups VIII and X to chromosomes in *Mus musculus* and identification of the centromeric end of linkage group I. Cytogenetics **10:** 452–464.

Schreck, R. R., D. Warburton, O. J. Miller, S. M. Beiser and B. F. Erlanger, 1973 Chromosome structure as revealed by a combined chemical and immunochemical procedure. Proc. Natl. Acad. Sci. U.S. **70:** 804–807.

Sun, N. C., E. H. Y. Chu and C. C. Chang, 1973 Staining method for banding patterns of human mitotic chromosomes. Mammalian Chromosome Newsletter **14:** 26–27.

Weisblum, B. and P. de Haseth, 1972 Quinacrine—a chromosome stain specific for deoxyadenylate-deoxythymidylate-rich regions in DNA. Proc. Natl. Acad. Sci. U.S. **60:** 629–632.

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