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CHROMOSOMAL BANDING PATTERNS OF TWO PARACENTRIC INVERSIONS

IN MICE

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Abstract. We have used banding techniques to study the chromosomes of mice heterozygous for two genetically defined paracentric inversions, In(1)lRk and In(2)5Rk. The banding patterns of the inversion-carrying chromosomes were altered as expected, and the relative lengths of the genetically and cytologically defined segments were comparable.

Introduction

With the advent of techniques which bring out banding patterns in mammalian chromosomes, it has been possible to characterize and distinguish whole chromosomes and parts of chromosomes. We have used these techniques to study paracentric inversions in mice established by other cytological and genetic methods (Roderick and Hawes, 1970; Roderick, 1971). Our objective is to correlate evidence about inversions obtained through cytological and genetic methods. This paper is a report of our cytological demonstration of two inversions, In(1)lRk and In(2)5Rk, for which chromosome location was already known.

Materials and Methods

Nine males heterozygous for In(1)lRk and five males heterozygous for In(2)5Rk were studied. In each case heterozygosity for the inversion was established by the frequency of first meiotic anaphase bridges. Although modifications were necessary, the techniques for producing chromosomal banding patterns were based on published methods (Miller et al., 1971; Nesbitt and Francke, 1973; Wurster, 1972; Sun et al., 1973). Standard air-dried bone marrow preparations were used for both methods. For fluorescent banding patterns fresh slides were stained 10 min with a 50 µg/ml solution of quinacrine mustard dihydrochloride, rinsed 15 to 20 min in running tap water and coverslipped in tris maleate buffer (pH 5.6). Bands were obtained only after sufficient washing to remove all excess stain. Preparations for Giemsa banding were heat-dried 6-24 hr at 65° and incubated 10 min in a potassium phosphate buffer (pH 6.8, 56°C) just prior to

enzyme treatment. Slides were either treated horizontally with a 0.025% solution of trypsin (Difco) or incubated in 0.05% α -chymotrypsin (Bovine pancreatic Type I, Sigma) at 37° for 7 min. Both enzymes were made up in Ca^{++} - and Mg^{++} -free Earle's saline. The slides were rinsed rapidly in two changes of the same saline and stained 5 min in 4% phosphate-buffered Giemsa. Photographs were made using Kodak High Contrast Copy film for Giemsa bands and Kodak Tri-X for quinacrine bands. Our designations of bands in this paper are those proposed by Nesbitt and Francke (1973).

The inverted segment In(1)lRk can be seen in Figure 1. The characteristically bright central band (1E) is shifted toward the centromere and the negative band (1B) at the centromere end is widened, making inverted chromosome 1 superficially resemble chromosome 2. The break points, of the inversion appear to be at the distal side of the most proximal negative band (1B) and inside the negative band (1F) just distal to the central bright band.

The definition of the inverted segment In(2)5Rk is not as striking (Figure 2). The characteristic negative band (2B) of chromosome 2 is not involved in the inverted segment. Distal to this band are two other major negative regions (2D and 2F). In the normal chromosome 2 the more distal (2F) is the wider of the two; in the inversion chromosome, the more proximal band (2D) is wider. This places the breakpoints within the two negative bands with a portion of the distal band contributing to the widening of the proximal one, or at the outer edges of each band or just beyond, with a complete substitution of the two bands. The latter seems

more likely since In(2)5Rk is similar in bridge frequency and genetic length to In(1)1Rk and the longer segment is comparable in size to that seen in In(1)1Rk.

We made attempts to compare the relative physical lengths of each inversion with their relative genetic lengths. Composite diagrams for each inversion and its chromosome were made (by M.T.D.) based on several different photographs of each. The physical lengths of each inversion relative to its respective chromosome were calculated from the composite diagram. Then, independently (by T.H.R.) the best estimate of the genetic lengths of each inversion were compared with the estimated length of the chromosome. The genetic length of the chromosome was derived from the estimate of the total genetic length of the mouse, 1200 cM (Searle et. al., 1970; Henderson and Edwards, 1968), multiplied by the percentages of the total physical length of each chromosome from actual chromosomal measurements (Committee, 1972). The genetic length of each inversion was derived from the maximum distance between those loci which do not recombine in the presence of the inversions (Roderick, Hawes, and Davisson, in preparation).

For In(1)1Rk, the percentage of physical length was 42% and the percentage of genetic length was 37%. For In(2)5Rk the respective percentages were 37% and 34%. With the admitted possible errors involved in each estimate, these percentages are in good agreement, and provide evidence that the physical lengths and genetic lengths are comparable at least over these segments of these chromosomes.

Discussion

Pericentric inversions are presumed to exist when there is a shift in the relative lengths of arms of a specific chromosome without any alteration in the total length of any chromosome or change in chromosome number. Although many such alterations are probably pericentric inversions, there are other possible explanations (Duffey, 1972) which should encourage caution. In some cases in humans (for example Ferguson-Smith, 1967, and Wilson et al., 1970), further genetic and cytogenetic evidence has confirmed the presence of an inversion. Adding further, the techniques for banding chromosomes have been very important additional evidence (Evans et al., 1971; Hauksdóttir et al., 1972).

Our characterization of paracentric inversions in mice has utilized evidence from (1) anaphase bridge frequencies which are consistent for specific inversions and significantly above the normal background frequency, (2) the transmission of the specific frequencies in the normal genetic expectations, (3) the lack of high anaphase bridge frequencies in inversion-homozygotes, (4) the lack of recombination of genes within the inverted segment both with each other and with the inversion (Roderick, 1971; Roderick and Hawes, 1970, 1973).

The fact that these inversions can be detected cytologically using banding techniques, suggests using these techniques as a possible alternative means for screening for paracentric inversions both in man and in experimental mammals.

With the advent of techniques for showing banding patterns, it became possible to assign linkage groups of the mouse to specific chromosomes

(Miller and Miller, 1972). Further application of these methods to mouse translocations has helped to establish the relative position with respect to the centromere of some individual genes and the location of certain genes near break-points of translocations (Miller et al., 1971, 1972, Francke and Nesbitt, 1971; Nesbitt and Francke, 1973). Inversions which can be characterized by these methods will be additionally useful locating genes near breakpoints. Also they will be useful in relating whole segments of the genetic map to the physical dimensions of the chromosome.

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Fig. 1. Multiple copies of Chromosomes 1 through 5 from animals heterozygous for In(1)1Rk. The inverted segment is designated by black bars.

Fig. 2. Multiple copies of Chromosome 1 through 5 from animals heterozygous for In(2)5Rk. The inverted segment is designated by bars.

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