Robertsonian fusion leading to the formation of stable dicentric chromosome in an ascites cell line of the mouse*

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Summary. The dicentric nature of a marker metacentric chromosome originated by robertsonian fusion has been established in the ascites cells of mouse sarcoma 180. C-banding analysis has revealed that the metacentric is actually a dicentric with 2 closely situated C-positive heterochromatic zones. The nature of the centromeres and the NF value of the cell indicate that this meta-dicentric marker has originated by breakage and fusion within each of the short arms of 2 acrocentric chromosomes.

Robertsonian translocation which involves the fusion of 2 rod chromosomes to yield 1 metacentric has been the subject of extensive studies in recent years, particularly after the introduction of the term by Matthey[1]. The phenomenon has largely been documented both in natural populations, as well as in various tissue culture lines. In most cases it has been assumed that robertsonian rearrangement involves the fusion of 2 acrocentric chromosomes following breakage proximal to the centromere in the long arm of one chromosome and simultaneous breakage in the short arm of the other[2,3], or due to the fusion between partly broken or eroded centromeres of 2 telocentric chromosomes[4–6]. Another type of rearrangement has also been documented in which fusion takes place following breakages in each of the short arms of 2 acrocentric chromosomes; the metacentric thus produced is actually a dicentric. The occurrence of such dicentric chromosomes has been reported in man[7,8] and in bull[9]. Recently, in course of our investigation on the chromosomes of an ascites cell line of mouse, an interesting incidence of robertsonian fusion leading of the formation of a stable 'meta-dicentric' chromosome has been recorded.

The ascites form of mouse sarcoma 180 (MS 180) is a significant tool in cytological research, because it grows very rapidly and can easily be maintained by serial in vivo transplantation in albino swiss mouse Mus musculus. The chromosomes of this ascites tumour were prepared by following the standard schedule of 0.1% colchicine-hypotonic saline citrate-acetic alcohol-flame drying technique. C-banding was performed by slight modification of the method suggested by Sumner and Evans[10]. One exception being that the flame-dried slides were kept in 0.02 N HCl (instead of 0.2 N HCl) at 27 °C for 1 h.

The modal chromosome number of this hypotetraploid ascites tumour is 75 (NF = 76) with a distinct bi-armed marker (figures 1 and 3). In most of the early metaphases, or in socalled prometaphases, this marker metacentric showed an extended primary constriction which led us to assume that this chromosome is probably a dicentric one with 2 closely placed centromeres. In order to confirm our assumption, C-banding analysis was made and it was revealed that the chromosome in question is actually a

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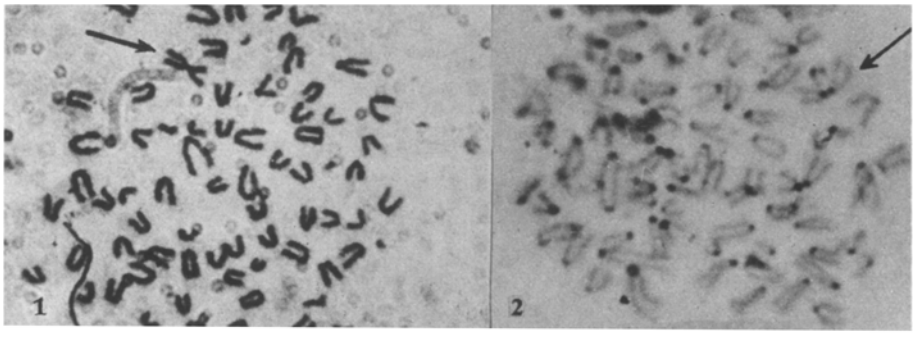

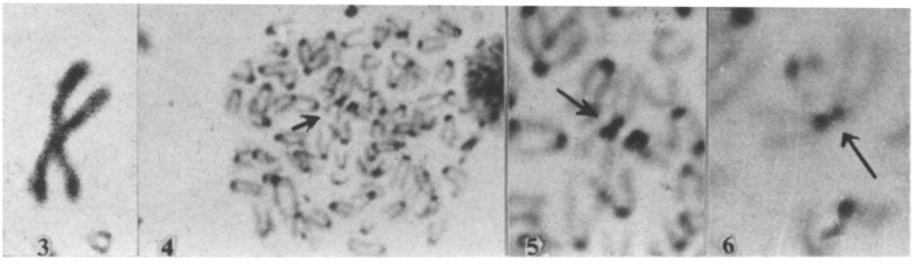

Fig. 1. Metaphase complement of mouse sarcoma 180 cell with a marker metacentric chromosome (arrowed).

Fig. 3. Enlarged view of the marker metacentric of MS 180.

Fig. 2, 4–6. C-banded metaphases of MS 180 showing double banded marker chromosome (arrowed).

dicentric with 2 closely located prominent C-positive heterochromatic zones (figures 2, 4–6). The existence of this meta-dicentric marker chromosome has been documented unmistakably in all the metaphases examined from different individuals after several successive in vivo passage of the tumour. The position and size of the centromeres and the NF value of the cell are indicative of the fact that this meta-dicentric chromosome has arizen by breakage and fusion within each of the short arms of 2 acrocentric chromosomes and constitutes a regular member in the karyotype of this ascites tumour.

Previously it was generally accepted that the dicentric chromosomes are usually unstable[3]. But evidence is now accumulating on the spontaneous(?) occurrence of stable and transmissible dicentric chromosomes in natural populations as well as in different continuous cell lines. Recently an extensive study on the dicentric nature of the chromosomes with multiple heterochromatic bands has been made in some continuous mouse cell lines by Chen and Ruddle[11], and in continuous human lymphoid cell lines by O'Neill and Miles[8]. Long before the discovery of differential centromeric staining, the dicentric nature of metacentric chromosomes was also suspected by Manna[12] when he was working with LP 59 cell line. More recently the existence of a stable dicentric resulting from Y-Y translocation has been reported in man by Ghosal et al.[13]. In addition to the previous reports, our present finding on the occurrence of a stable and transmissible dicentric in MS 180 cells will add further cytological data to the problem of the stability of dicentric chromosomes.

Considerable controversy still exists regarding the exact nature of rods in mouse. After an extensive study on the chromosomes of a large variety of animals, White and others have adopted the view that all naturally occurring rods are acrocentric[3]. But recently, from the whole mount EM study, Comings and Okada[14] have concluded that the rods of mouse, sheep and goat are all telocentric with no evidence for a short arm. In our previous report[6] on the fusion metacentric of the house mouse of Asian variety, we have concluded that the rods of the mouse are all telocentric in nature. But the nature and placement of centromeres in the meta-dicentric marker of MS 180 cell line lead us to suggest that the chromosomes of this particular tumour are acrocentric in nature.

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Solanum verrucosum as a base for mutation breeding in potato

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Summary. Solanum verrucosum Schlechtd. is proposed as a base for mutation breeding in potato. Its mutability was demonstrated with N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea as mutagens.

The induction of mutations in a number of cultivated plants has become a valuable part of breeding programs, but not for the cultivated potato, Solanum tuberosum. This species has more than 100 wild relatives in Central and South America, which offer a large reservoir of potentially useful genes. Some of these have been used in breeding for resistance to Phytophthora infestans, virus diseases, frost, nematodes and insects[2,3]. However, the transfer of genes from some wild species to S. tuberosum is often difficult or impossible due to cross-incompatibility, sterility and different levels of ploidy. Additionally, some traits which would be most useful for potato improvement (e.g. leaf roll immunity) are not known to be present in any of the wild or cultivated Solanum species. Thus mutation breeding represents the only way to obtain new characteristics, as well as to reproduce existent characteristics which are not transferable from other species. However, mutation breeding in S. tuberosum presents some serious difficulties. The species is tetraploid (2n = 48), and therefore the probability of having recessive gene mutations expressed is low. Moreover, identification and isolation of a mutation from the highly heterozygous segregating progeny is very difficult. Therefore mutation experiments undertaken with S. tuberosum have involved only vegetative progenies of the M_1-generation, while seed progenies have not been previously fully analyzed[4–8]. To avoid these difficulties, a diploid Solanum species or derivative could be used as a base for mutation induction in potato. Theoretically then, such induced mutations could be introduced into the S. tuberosum genome. This would open new possibilities in potato breeding, which has been limited until now to crossing, selection, and the appearance of somatic mutations. The use of 'dihaploids' (2n = 24), derived from S. tuberosum, or allied diploid

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