CYTOGENETIC RESEARCHES IN HAMSTERS

Des recherches cytogénétiques chez les hamsters

Cytogenetische Untersuchungen beim Hamster

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The hamster became lately first order laboratory animals because of some important characteristics: they can be easily bred and multiplied, they reach soon the maturity, they can be inbred, they give lines of a high homogenesis level, and they have chromosomes fit for the cytogenetic researches.

In our country beginning with the year 1968 we have made thorough regarding many aspects at the species of hamsters *Mesocricetus auratus, M. newtoni, M. brandti,* and we have also made studies of the sex chromatin at *Cricetus cricetus* and *Cricetulus griseus.* These researches were completed with studies on some interspecific hybrids obtained in the nursery animals laboratory of the Department of Genetics, University of Bucharest, taking into account the fact that the hybrids animals present a great importance for the immunology studies, for the researches regarding the chromosomes homology and for the karyotype evolution.

The methods and the techniques used were: air dried method for obtaining the chromosomes preparations after the colchicine injection of the animals in order to make the karyotype and idiogram and for the study of the cromosomes distribution in the metaphases plates; autoradiography by tritiated thymidine labelling of the chromosomes, in order to study the mode of the chromosomes replication; the method of the chromosomes bandization by denaturation-renaturation of the genetic material with proteolitic substances or with the temperature in order to established the chromosomes bands pattern; and different methods for the obtaining of the sex chromatin staining with violet cresyl and other substances.

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The researches made at the Roumanian hamster (*Mesocricetus newtoni*) proved this hamster is a separate species having $2n = 38$, infirming the previous opinion of other authors which have considered the Roumanian hamster as a subspecies of the golden hamster (P. RAICU *et al.*, 1968). The karyotype is made of 18 pairs of autosomes and one pair of sex chromosomes, at female the X chromosomes being one of the biggest submetacentrics and at male the Y chromosome is a metacentric.

Using an original method we have statistically studied the chromosomes distribution in the metaphase plate. Basing on these researches we have demonstrated that at least a part of the chromosomes has a non-random distribution in the metaphase plate and respectively in the cell. The chromosomes of the pair 3 and 9 and the sex chromosomes too, present a peripheral localization in the metaphase plate, while the distribution of other chromosomes (the pairs 16, 17 and 18) is non-peripheral (P. RAICU *et al.*, 1968).

We have demonstrated the correlation between the peripheral localization of one of the X chromosomes at female, its late replication and the formation and the appearance of the sex chromatin. This X chromosome has an asynchronous replication comparatively to its homologue and to the autosomes. Generally, we have observed an asynchronous replication both at the interchromosomal and at the intrachromosomal level. The existence of some autosomes with the both arms completely unlabelled at the end of the S phase, suggests that these proceeded from the fusion of some acrocentrics from the golden hamsters manifesting a similar labelling pattern (P. RAICU *et al.*, 1972). The sex chromatin incidence is significantly greater at female than at male. The presence of the second Barr corp with half of size of the first one, was explained by its origin by the heterochromatinization, heteropicnosis and the genetic inactivation of one half of the other X chromosome at female (P. RAICU *et al.*, 1970). This is due to the fact that at the Roumanian hamster too, the X chromosome has double sizes comparatively to other mammalian species, representing about 10 % of genome. In the male meiosis we have observed end-to-end type association of the sex chromosomes X Y.

The biometrical study of the chromosome complement at the Kurdistan hamster (*Mesocricetus brandti*, $2n = 42$) shoved that it is made of 20 pairs of autosomes with the lenght varying between 2.84 and 10.03 μ and the heterosomes are represented by a metacentric X chromosome of big size (9.02 μ) and by a submetacentric Y chromosome of middl sizes (7.15 μ) (P. RAICU *et al.*, 1973). The autosomes are submetacentrics excepting the pair 17 which is made of metacentrics. The biometrical study and the idiogram permitted the accurate establishing of the chromosomes types. So, we could demonstrate, for instance, that the pair 20 is made of submetacentric chromosomes with an arm ratio 2.44. This observation infirmed the studies of J. M. LEHMAN *et al.* (1967) and of N. B. TODD *et al.* (1972) which supported the opinion that the chromosomes of the pair 20 are acrocentrics. The meiosis study showed that in the diakinesis and methaphase I 21 bivalents of different types are present. The sex chromosomes present a similar association to that of the heterosomes in the Roumanian hamster, namely end-to-end type, where heterosomal segments are present. The sex chromatin studied in three organs (liver, kidney, spleen) is much more frequent at female and much easier to study in liver because the nuclei are less deformed

and permitted with a greater accuraty the evidence of Barr corps. It seems that at this species too the X chromosome is duplicated.

Our results regarding the hamster species *Mesocricetus auratus* are very similar to those in the literature. The obtained bands C permitted us to conclude that the constitutive heterochromatin fully participates to the formation of the Y chromosome and partially of one of the X chromosomes. The association of the non-homologue chromosomes in meiosis is also of end-to-end type for the heterochromatic segments. The high incidence of the sex chromatin at female has been correlated with this type of association and with the mode of formation of the heterosomes.

The study of the sex chromatin at the species *Cricetus cricetus* ($2n = 22$) and *Cricetulus griseus* ($2n = 22$) showed the absence of a nuclear sex dimorphism given by an equal proportion of the sex chromatin in the both sexes. At the some time the presence in a greater number of some nuclei with only one sex chromatin supported the hypothesis of its origin from a single X chromosome at female. In the favour of this hypothesis is also the side-by-side sex chromosomes associations because of the high dregree homology of the respective heterochromatic segments (P. RAICU *et al.*, 1970).

The study of the hybrid obtained from reciprocal crosses between *M. newtoni* and *M. auratus* showed that the Romanian hamster is a separate species and it is not a subspecies of the golden hamster. The karyotype of this hybrid showed a chromosomal complement ($2n = 41$) resulting by the totalyzing of the haploid sets chromosomes of the genitors. The total sterility both of the females and of the males, the high frequency of prophase I and the small number of metaphase I, the presence but of the univalents because of the lack of the homology between the chromosomes of the two genitor species demonstrate that the genitors are distinct species (P. RAICU *et al.*, 1969).

The interspecific hybrid between *M. brandti* ♀ and *M. auratus* ♂ presents an intermediate number of chromosomes ($2n = 43$) comparatively to the genitor species This hybridization was very difficult to be realized and the number of offsprings was very small. Althoug we tried many times to obtain the reciprocal hybrid, we could not succeeded it. In the meiosis hybrid *M. brandti* ♀ × *M. auratus* ♂ we could identify only prophases I, diakinesis and metaphases I. We explaines the absence of subsequent stages by the lack of homology between the chromosomes of the two species, so that it is not possible to form bivalents. Consequently, the hybrids are whole sterile. The morpho-histological studies explain this sterility as at the respective hybrids the spermatogenesis is absent. These researches demonstrated that those two species are distinct and separate too (P. RAICU *et al.*, 1973).

In the interspecific hybrid between *M. brandti* ♀ and *M. newtoni* ♂ ($2n = 40$), we have observed a high degree of sterility which has been explained by the presence of the multivalents in diakinesis and metaphase I, which determine a non-balanced disjunction of the genetic material. So, we have identified 16 bivalents and 2 tetravalents in the male meiosis that what showed a high degree of homology between the chromosomal sets of the two species (P. RAICU *et al*, 1972).

The sex chromatin study at the three hybrides was correlated to the study of the sex chromosomes at female, and the genitor species from which the X chromosome proceeded.

The cytogenetic researches of these hybrids permitted us to conclude, basing on the homology degree between the chromosomes of the two sets of the complement, that the three species are well individualized and distinct and that they have well differentiated karyotypes. All the three species are related to them but it seems that *M. brandti* is more close to *M. newtoni* than to *M. auratus*. We would consider that the speciation process has occurred by a Robertsonian fusion mechanism of some chromosomes from *M. auratus*, by this way resulting the two other species.

RESUME

Les auteurs ont effectué des recherches cytogénétiques chez différentes espèces des hamsters et chez quelques hybrides interspécifiques. Nos recherches permettent de conclure que le hamster roumain (*Mesocricetus newtoni*) possédant $2n = 38$ et un caryotype different de *M. auratus*, ne peut être considéré la sous espèce de ce dernier, mais au contraire une espèce valable. A l'aide d'une méthode originale nous avons remarqué qu'au moins une partie des chromosomes chez le hamster roumain ont une non-random distribution dans la plaque métaphasique.

En ce qui concerne le hamster de Kourdistan (*M. brandti*, $2n = 42$) nous avons établi avec exactitude le caryotype et l'idiogramme.

En fin les auteurs ont etudié la mitose et la méiose chez quelques hybrides interspécifiques du genre *Mesocricetus*. Tous les hybrides possèdent des characteristiques chromosomiques intermediaires entre les géniteurs et sont tout-à-fait stériles. On peut conclure que les trois espèces sont valables et bien individualisés.

Tous les trois espèces de *Mesocricetus* etudiés présentent un dimorphisme nucléaire sexuel, tandis que chez les espèces *Cricetus cricetus* et *Cricetulus griseus* le dimorphisme sexuel est absent.

ZUSAMMENFASSUNG

Verfasser führten cytogenetische Untersuchungen bei verschiedenen Hamsterarten und einigen interspeziefischen Bastarden durch. Dabei stellte sich heraus, dass der rumänische Hamster keine Subspezies des Goldhamsters ist, sondern eine selbstständige Art darstellt. Beim rumänischen Hamster wurde eine nicht zufällige Verteilung einiger Chromosomen in den Methaphase Platten beobachtet.

Karyotyp- und Idiogrammbestimmungen beim kurdistanischen Hamster führten zu neuen, bzw. von Literaturangaben abweichenden Ergebnissen, die untersuchten Bastarde wiesen im Vergleich zum Elternpaar intermediäre Chromosomcharakteristiken auf, in dem sich alle durch Sterilität auszeichneten. Untersuchungen über Sexualchromatin zeigten, dass alle Arten der Gattung *Mesocricetus* durch einen geschlechtlichen Kerndimorphismus ausgezeichnet sind und, dass dieser bei den überen Arten abhanden ist.

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