A comparative study of the chromosome banding patterns of Mesocricetus newtoni and Mesocricetus auratus

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Introduction

The significance of karyotype analysis for establishing phylogenetic relationships within or between different taxa has been clearly demonstrated, especially through the investigations, amongst others, by MATTHEY (1961, 1964), NADLER (1969) and Hsu and ARRIGHI (1966). Nevertheless, the restrictions of the techniques available have not always yielded conclusive results. Some attempts have been made to recognize homologies between the chromosomes of related species using idiograms. The most recent reports were those by FREDGA (1972) for mongooses (Viverridae), and by TODD et al. (1972) for three hamster species within the genus *Mesocricetus*.

The recent special staining techniques (C-, G- and Q-banding) allow a superior identification of homologous chromosomes, and the individual chromosome pairs of every species can be differentiated. On the other hand, the chromosomal changes having occurred during evolution may be traced. Therefore, it may be possible to throw more light upon the mechanisms which determine the actual configuration of the karyotype of a particular species. Under this aspect we have performed a comparative study of the chromosome banding patterns of two hamster species belonging to the genus *Mesocricetus*.

The karyotype of the Syrian hamster (*Mesocricetus auratus* 2n = 44) is well established (GALTON and HOLT 1964; LEHMAN et al. 1963; FREDGA and SANTESSON 1964; SCHMID 1967; HSU and ARRIGHI 1971).

RAICU and BRATOSIN (1966) und RAICU et al. (1968) described the karyotype of the Rumanian hamster (*Mesocricetus newtoni*), having a diploid complement of 38 chromosomes. Recently the distribution of constitutive heterochromatin and the chromosomal banding pattern of this species has been reported by us (VOICULESCU et al. 1972).

It is attempted here to establish the degree of concordance in the chromosomal banding patterns, and to discuss the possible mechanisms involved in karyotype differentiation of these two species.

Material and methods

The Rumanian hamster is a wild living animal of South-Eastern Rumania (The province Dobroudja) and Bulgaria, occuring in a very restricted location. The specimens used in this study were kindly supplied by Prof. P. RAICU and Dr. L. MANOLACHE, Bukarest.

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Bone marrow air-dryed and flame-dryed chromosome preparations were produced for

utilization of the C-staining and G-staining method, respectively. The special staining for constitutive heterochromatin was performed according to the method described by ARRIGHI and Hsu (1971).

Chromosomal G-banding patterns were obtained using the procedure described by SCHNEDL (1971) with some modifications. The Giemsa staining was made according to CHAUDHURI et al. (1971).



Fig. 1. Karyotype of the Rumanian hamster (M. newtoni). a = G-banding pattern; b = C-banding pattern



Fig. 2. Karyotype of the Syrian hamster (M. auratus). a = G-banding pattern; b = C-banding pattern

Results

The chromosomal G-banding patterns of the two hamster species are illustrated in figs. 1a and 2a respectively. Since it will be demonstrated that the difference in fundamental number (FN) of these two species is due to differences in the distribu-

tion of constitutive heterochromatin, we present the localization of heterochromatin within he karyotypes in figs. 1b and 2b respectively, as obtained by the C-staining technique. In order to clearly identify the chromosomes of the Syrian hamster carrying entirely heterochromatic short arms, we have selected metaphases stained by the C-banding technique which also exhibited the G-banding pattern to a certain degree. In the Rumanian hamster this was not necessary since those particular chromosomes had already been identified in our recent report (VOICULESCU et al. 1972). Fig. 3 shows the chromosomes of both species arranged in such a way that the banding patterns correspond to each other, indicating their presumed homology.

Taking into account that the Rumanian hamster has a lower number of chromosomes than the Syrian hamster, and that all the chromosomes are biarmed, the chromosomal set of this species is used as the basis for comparison. In the following we describe the homologies of the banding pattern between the chromosomes or chromosomal segments of the two species, as illustrated in fig. 3.

Mesocricetus newtoni	Mesocricetus auratus
Chromosome 1: — short arm	long arm of chromosome 1
– long arm	long arm of chromosome 3

The short arms of chromosomes 1 and 3 of the Syrian hamster are heterochromatic (C-staining, fig. 2b). A very characteristic feature of chromosome 1 of the Syrian hamster is the presence of an unstained zone on the long arm near the centromere. This feature is also present on the short arm of chromosome 1 of the Rumanian hamster.

Chromosome	2:	_	short arm
		_	long arm

acrocentric chromosome 19 acrocentric chromosome 17

This is a typical example of centric fusion of the Robertsonian type.

Chromosome 3: - short arm

long arm

long arm of chromosome 12 acrocentric chromosome 16

The short arm of chromosome 12 of the Syrian hamster is heterochromatic (Cstaining, fig. 2b).

Chromosome 4:

It can be considered as homologous to chromosome 7 of the Syrian hamster. The different centromeric index is due to an additional telomeric heterochromatic band on the short arm of chromosome 4 of *M. newtoni*.

Chromosome 5:

The banding patterns correspond satisfactorily.

The short arm is longer in chromosome 5 of the Rumanian hamster, which results in a different cetromere index. The C-staining proves that the short arms of both chromosome pairs are heterochromatic.

Chromosome 6:

Chromosome 5

An unstained zone on the short arms near the centromeres is a characteristic feature of these chromosomes.

Chromosome 7:

Chromosome 15

The banding patterns prove the homology of these Chromosomes. The short arm of chromosome 7 (M. newtoni) has an additional telomeric heterochromatic band which results in a different centromeric index.

Chromosome 8:

Chromosome 8

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Fig. 3. Karyotypes of Rumanian and Syrian hamsters arranged in columns to show their presumed homology. R = Rumanian hamster; S = Syrian hamster

Chromosome 9:

Chromosome 9

In the Rumanian hamster chromosomes 8 and 9 have a larger unstained region on the long arm near the centromere than the chromosomes 8 and 9 from the Syrian hamster.

Chromosome 10:

Perfect homology exists between these two chromosomes. In the Rumanian hamster the short arm of chromosome 10 has an additional heterochromatic band at the telomere, emphasized by the C-staining (fig. 1b).

Chromosome 11:

A large unstained region between the central and distal third of the long arms, and a small one on the short arms near the centromeres of both chromosomes are characteristic features. The short arms are unequal that of chromosome 2 being longer than that of chromosome 11, therefore the centromeric indices are different. The C-staining shows the short arm of chromosome 2 (Syrian hamster), and a telomeric band on the short arm of chromosome 11 (Rumanian hamster) to be heterochromatic (figs. 1b and 2b).

Chromosome 12:

- short arm heterochromatic (fig. 1b)

— long arm

Chromosome 13:

- short arm heterochromatic (fig. 1b)

— long arm

- whole chromosome 13

- acrocentric chromosome 18

Regarding chromosome 13 of the Syrian hamster, we presume a translocation of the short arm to the long arm (pericentric inversion) resulting in the long arm of chromosome 13 of the Rumanian hamster; then, banding patterns are in accordance with those of the long arm of chromosome 13 (Rumanian hamster) (fig. 4).

Chromosome 14:

- long arm - long arm of chromosome 14 The short arms are heterochromatic (Cstaining). On the long arm of chromosome 14 of the Rumanian hamster an unstained zone near the centromere exists resulting in a different centromeric index.

Chromosome 15:

- long arm - long arm of chromosome 11 The short arm of chromosome 15 is heterochromatic (fig. 1b) and longer.

Chromosome 16: Chromosome 10

Chromosome 17:

As in the chromosome 14 of the Rumanian hamster, the long arm of chromosome 17

exhibits an unstained zone near centromere, resulting in a different centromeric index.

Chromosome 18:

Chromosome X:

There is good homology in the euchromatic segments, which posses two characteristic bands each. This banding pattern seems to be a general feature of the eutherian X-chromosome. We have observed an identical banding pattern in *Microtus agrestis* and in *Mus musculus*.

Chromosome Y:

In both species the Y-chromosomes are heterochromatic and show no banding pattern.



Fig. 4. Pericentric inversion on chromosome 13 of the Syrian hamster (S 13); the corresponding bands are located in the long arm of chromosome 13 of the Rumanian hamster (R 13).

Probably chromosome 21

Chromosome 2

Chromosome 6

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Discussion

In a recent report YERGANIAN (1972), refering to the species Mesocricetus auratus (2n = 44), Mesocricetus brandti (2n = 42) and Mesocricetus newtoni (2n = 38) suggested that: "one cannot judge whether the trend in speciation followed a numerical increase, i. e., 38-42-44, or numerical decrease, i. e., 44-42-38 in the number of chromosomes".

Indeed, not even the banding methods offer more precise clues to this question. The analysis of the banding pattern reveals only the extent of concordance, and not the trend of speciation in the two species examined here. Arbitrarily, we consider the karyotype with 2n = 44 as the ancestral one. In favour of this assumption is the occurence of one Robertsonian translocation, two accrocentrics of the Syrian hamster forming one metacentric of the Rumanian hamster. However, this as well as the other mechanisms discussed subsequently, can also be interpreted conversely. A more comprehensive study including all the species that belong to the genus *Mesocricetus*, could possibly give closer information on the trend of speciation within this genus.

The results of our study demonstrate a good correspondence in the banding patterns either of whole chromosomes, or of chromosomal segments of the Rumanian and Syrian hamsters.

Refering to the possible mechanisms that occured during speciation of hamsters, WURSTER et al. (1971) supposed that Robertsonian fusions played an insignificant part, inversions and translocations being more important. Our results confirm this opinion. We identified only one translocation of the Robertsonian type, i. e. the metacentric chromosome 2 of the Rumanian hamster resulting from a centric fusion of the acrocentric chromosomes 19 and 17 of the Syrian hamster. In another two presumptive translocations which resulted in the chromosomes 1 and 3 of the Rumanian hamster, the long arms of two submetacentrics, and the long arm of a submetacentric together with an acrocentric chromosome were involved respectively. The C-staining proved that the short arms of all the 3 submetacentrics participating in translocations are heterochromatic. Therefore, from these 3 translocations six heterochromatic short arms remain in the Syrian hamster, while being unpaired in the Rumanian hamster.

The fundamental number (FN) is 80 for the Syrian hamster and 76 for the Rumanian hamster. WURSTER et al. (1971) gave a FN = 78 for the Syrian hamster, probably because they considered chromosome pair no. 21 as being uniarmed. Since in the Rumanian hamster the chromosome pair no. 18 is alike and probably homologous to no. 21 of the Syrian hamster, we consider both chromosome pairs to be biarmed, giving a FN = 80 for the latter.

Taking into account that chromosome 12 of the Rumanian hamster has a heterochromatic short arm in contrast to the corresponding chromosome 18 of the Syrian hamster (which is acrocentric), only four of the six heterochromatic short arms in the Syrian hamster mentioned above are left, resulting in the difference in the FN of the two species. The resumed pericentric inversion of chromosome 13 of the Syrian hamster, resulting in the long arm of chromosome 13 of the Rumanian hamster, does not modify the FN, since, in addition, chromosome 13 of the Rumanian hamster has a heterochromatic short arm.

Our analysis revealed the presence of additional telomeric heterochromatic bands on the short arms of some of the chromosomes (the chromosomes 4, 5, 7, 10, 15 of M. newtoni compared to 7, 4, 15, 6, 11 of M. auratus respectively, and a longer short arm of chromosome 2 of M. auratus compared to the short arm of chromosome 11 of M. newtoni). From these observations, it could be concluded that of the four hete-

rochromatic short arms remaining from the above estimate, according to the FN two could correspond to the heterochromatic short arms of chromosome pair no. 13 of M. newtoni. On five chromosome pairs of the Rumanian hamster, additional heterochromatic telomeric bands exist in contrast to their correlates in the Syrian hamster; in the Syrian hamster only one chromosome pair (no. 2) possesses a supplementary heterochromatic band on its short arm, contrary to its correlate in the Rumanian hamster.

In conclusion, a rough assessment indicates that within the autosomes, the quantity of constitutive heterochromatin remains approximately constant, and the difference in FN is due to the presence of four supplementary heterochromatic short arms in the Syrian hamster; however, this heterochromatin is nevertheless present and can tentatively be located in the karyotype of the Rumanian hamster.

HSU and ARRIGHI (1971) assumed the addition of heterochromatin for the formation of new arms to be a possible mechanism of karyotype evolution in the Syrian hamster. Since both of the hamster species examined here have some chromosomes with entire heterochromatic short arms, this hypothesis does not contribute to the problem of the ancestral hamster genome.

In the sex chromosomes, the quantity of constitutive heterochromatin is different between these two species. It is known that the X-chromosome of *M. auratus* represents about $10,2^{0}/_{0}$ of the female haploid complement (SCHMID 1967). For the X-chromosome of *M. newtoni* we have estimated this figure to be $7,4^{0}/_{0}$ (VOICULESCU et al. 1972). It can be assumed that the euchromatic segments of the X-chromosomes in both species are of equal size and homologous having the same banding pattern. For the difference in length, therefore, a larger quantity of constitutive heterochromatin on the X-chromosome of *M. auratus* is responsible. The Y-chromosome seems also to be longer in the Syrian hamster.

Summary

The presumed homology between the chromosomes of Rumanian and Syrian hamsters is discussed. In general a good correspondence of the chromosomal banding patterns of these two species is found. The difference in fundamental number (FN = 76 in M. newtoni and 80 in M. auratus) is due to four additional heterochromatic short arms in the Syrian hamster. This heterochromatin nevertheless is present and can tentatively be located in the karyotype of the Rumanian hamster. It is concluded that within the autosomes, the quantity of constitutive heterochromatin remains approximately constant in both species. In this study translocations were found to be a frequent chromosomal change; amongst these, only one of the Robertsonian type was observed. The euchromatic segments of the X-chromosomes possess two characteristic bands and this banding pattern seems to be a general feature of eutherian X-chromosomes.

Zusammenfassung

Eine vergleichende Untersuchung über Bänderungsmuster der Chromosomen von Mesocricetus newtoni und Mesocricetus auratus

Die Frage der Homologie zwischen den Chromosomen des rumänischen Hamsters und des Goldhamsters wird untersucht. Die Bänderungsmuster auf den Chromosomen beider Species entsprechen sich weitgehend. Der Unterschied in der Anzahl Chromosomenarme (76 bei *M. newtoni* und 80 bei *M. auratus*) ist durch vier heterochromatische kurze Arme bedingt, die sich beim Goldhamster zusätzlich finden. Diese heterochromatischen Anteile lassen sich jedoch auch mutmaßlich im Chromosomensatz des rumänischen Hamsters nachweisen. Daraus ist zu schließen, daß der Anteil an konstitutivem Heterochromatin auf den Autosomen bei beiden Species etwa gleich ist. Weiterhin ließen sich eine Anzahl von Translokationen nachweisen, durch die sich die beiden Arten unterscheiden, darunter nur eine Robertsonsche Translokation. Die euchromatischen Segmente des X-Chromosoms weisen zwei charakteristische Bänder auf, die auch auf den X-Chromosomen anderer Plazentalier zu beobachten sind und daher ein generelles Merkmal darstellen dürften.

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