Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **83** (1989), n.1, p. 319–326.

Accademia Nazionale dei Lincei

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1989.

Atti Acc. Lincei Rend. fis.
(8), LXXXIII (1989), pp. 319-326

Genetica. — *Satellite DNA and chromosome translocations: a hypothesis regarding «Robertsonian» chromosome formation.* Nota di CARLO ALBERTO REDI (*), SILVIA GARAGNA (*) ed ERNESTO CAPANNA (**), presentata (***) dal Corrisp. E. CAPANNA.

ABSTRACT. — A hypothesis is presented concerning the formation of «Robertsonian» metacentrics, i.e. whole chromosomal arm translocations. During DNA-synthesis the base pairing of homologous parental strands, carried by different chromosomes, produces heteroduplexes. The rearranged DNA region would then be cut off by topoisomerases, and two acrocentric chromosomes would be fused into one metacentric. The analysis of the structure of the genome of different animal groups such as Muridae, Bovidae and Primates, supports the idea that chromosomes can exchange parts, or whole arms, in sites where base sequences show a high degree of homology.

KEY WORDS: Robertsonian translocations, Satellite DNA, *Mus*.

RIASSUNTO. — *DNA satellite e traslocazioni cromosomiche: un'ipotesi riguardante la formazione di metacentrici «Robertsoniani».* La fusione Robertsoniana (Rb) di cromosomi acrocentrici è uno degli eventi più frequenti capaci di diversificare il cariotipo. Ciononostante il meccanismo molecolare di tale evento non è ancora chiaro. Nella presente Nota viene suggerita l'ipotesi che, durante la sintesi del DNA, l'appaiamento di basi di sequenze omologhe su filamenti parentali 5′ e 3′ (di due diversi cromosomi le cui coordinate polari siano anti-rotate) porti alla formazione di un eteroduplex. Il taglio e la chiusura della regione di DNA riordinata (da parte di una topoisomerasi) unirebbe in un metacentrico i due cromosomi acrocentrici. L'analisi della organizzazione del genoma in relazione alla struttura del cariotipo in diversi gruppi animali da sostegno alla idea che i cromosomi possano scambiarsi parti, o intere braccia, in siti ove la sequenza di basi mostri un alto grado di omologia.

The formation of Robertsonian (Rb) chromosomes (whole chromosomal arm translocation processes or «centric fusions») is one of the main events able to cause karyotype diversification in mammals but, in spite of its wide occurrence, the mechanism is still obscure. The classic cytogenetic theories (fig. 1*a*) involve simultaneous breakages in the short arms of one acrocentric, and in the long arm of the other, leading to the production of a monocentric metacentric, with the concomitant loss of a small chromosomal fragment [1]. The direct way of testing this hypothesis is to detect changes in DNA content after Rb formation. There is some evidence for substantial DNA loss in some organism (e.g. man), although usually changes in DNA content cannot be proved, particularly for the house mouse of the Western European countries, (*Mus domesticus*), a species in which Rb chromosomes occur with very high frequency (not comparable with any other animal) with more than 116 Rb's identified in natural population, of the 171 possible combinations for fusion between 19 autosomes of the standard karyotype, and as many as 18 pairs of Rb chromosomes can be present in a single karyotype (reviewed by Capanna, 1985 [2] and by Redi and Capanna, 1988 [3]. For the house mouse, it has been proved that chromosomes are acrocentrics and that the most likely mode of Rb chromosome formation (accepting

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(***) Nella seduta dell'11 febbraio 1989.

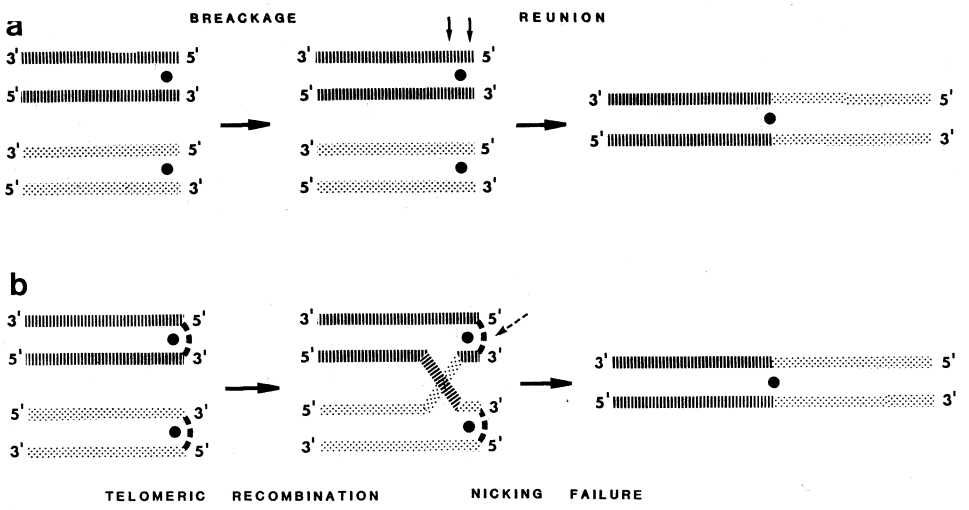

Fig. 1. – Existing hypotheses for Robertsonian formation: *a*) the classical model of breakage reunion events in the pericentromeric areas (John & Freeman [1]); *b*) mutations in terminal hairpin sequences (→) during telomeric recombination (before replication) that prevent restriction endonuclease nicking of the replication intermediate (Holmquist & Dancis [7]). The two hypotheses assume different theoretical requirements for their validity and different cytological factors to be involved in Rb formation triggering. The resulting probability of the formation of Rb chromosomes is itself very low.

breakage-reunion events) would involve simultaneous breakages in the short arms of two acrocentrics, with rejoining of the long arms into a Rb metacentrics and concomitant loss of a small acentric fragment (*i.e.*, mode number 4 of the John and Freeman classification [1]). Different methodological approaches for showing DNA losses have always failed, even in mice with as many as 18 Rb's in 40 chromosomes [4, 5]. Thus, the accepted conclusion is that if the DNA losses occur they must be of less than 100 000 bp ÷ 400 000 bp per acrocentric and involve short DNA sequences of highly repetitive satellite DNA.

Our increasing knowledge regarding the structure, function and molecular composition of centromere and telomere regions [6] and genome organisation in this last decade has led to other hypotheses about Rb formation. Holmquist and Dancis [7], analysing the problem of a telomere replication, suggested that the most reasonable molecular model for the resolution of the «Okasaki dilemma», i.e. a 5′ terminal gap replication, requires all telomeres in a cell to recombine via common DNA sequences before replication. Therefore they hypothesized a model of Rb translocation (fig. 1*b*) based on recombination between satellite DNA sequences on different chromosomes: if there is a failure of the enzyme machinery deputized to separate a pair of replicating telomeres, because of a mutation in a terminal hairpin sequence that could prevent recognition by the restriction endonuclease of the replication intermediate's palindrome, then two chromosomes could be joined and one of the two kinetochore organiser sequences could be inactivated. Stahl *et al.* [8], on the basis of ultrastructural studies of human chromosomes, showed overlapping of chromatids of nonhomologous acrocentrics in the nuclear region during meiotic prophase: they proposed that breakage-

reunion events could occur between nonhomologous chromatids, with monocentric and dicentric Rb chromosome formation depending on the position of the breakage-reunion. On the contrary, Miller *et al.* [9], applying the breakage-reunion hypothesis to determining the relative probability of a NOR-bearing chromosome being involved in an Rb chromosome, found that fusion of nucleoli cannot be the key factor predisposing to centric fusion in the mouse.

On the basis of these hypotheses, it is still hard to explain the differential rate with which Rb chromosomes arise in karyotypes of different species. It would seem reasonable to assume that breakage-reunions or mutations give the same chances to acrocentrics to fuse, in spite of the species karyotype to which they belong (but just compare the house mouse and the bull karyotypes, both of them with an all-acrocentrics constitution: in one species more than 100 different Rb chromosomes, in the other just few).

The increasing use of the house mouse model of chromosome variability in experimental genetics and medicine, and the extraordinary situation of chromosome diversification in the karyotype of this species, calls for a model less causative in its requirements and based on molecular events, the occurrence of which is related to inherent genomic traits. Theoretically, the two minimum requirements for Rb fusion are, i) a favourable spatial relationship between two chromosomes, and ii) an interaction between their DNA strands that will allow them to join. The favourable spatial relationship could be achieved on a stochastic basis, since the highly ordered structure of the different genomic portions in a nucleus already entails clustering of pericentromeric areas for coming together of functionally related genomic portions. For interaction, whatever the DNA structure formed immediately, it must entail that the DNA strands of the two chromosomes fit the polar co-ordinates in a suitable geometrical position, to allow the joining of the DNA backbone with the DNA sequences of the two chromosomal arms remaining centromerically oriented, since Rb chromosomes have the two DNA strands of the two chromosomal arms centromerically oriented as they are in the acrocentric structure.

To see how Rb fusion can occur, we have considered the house mouse karyotype not only because of its high frequency of Rb fusion, but because some cytoarchitectural properties of its genome are well known: it has 40 acrocentric chromosomes which centromeres tend to cluster *in vivo* [10] and thus the chromosomal areas directly involved in Rb formation are in proximity, or overlap, to provide a favourable spatial relationship for Rb fusion (see fig. 2 *a* and *b*).

Moreover, the satellite DNA sequences have been studied in detail and their chemical composition, structural features and chromosomal allocation are one of the best known among animals. What is mainly relevant in our regard is that these sequences are highly homogeneous (as compared with other organisms) and all of the 40 acrocentrics, except the *Y*, have quite similar satellite DNA sequences in the pericentromeric areas [11, 12, 13]. This cytoarchitectural situation means that one can reasonably expect similar DNA sequences of several chromosomes to be intermingled and overlapping, at least at some moments during the cell cycle. Now, even though at the present time the structural relationships among the origin of replication in satellite DNA, the centromere itself and the array of the satellite are not known, it can be

assumed with some confidence that partially replicated regions of DNA with discontinuous DNA synthesis will produce unpaired single strand regions. It is evident from trials with DNA models that these single strand regions can interact through base-pairing between complementary satellite DNA sequences of two different chromosomes: a DNA sequence on a 5′ parental strand could base-pair with a homologous

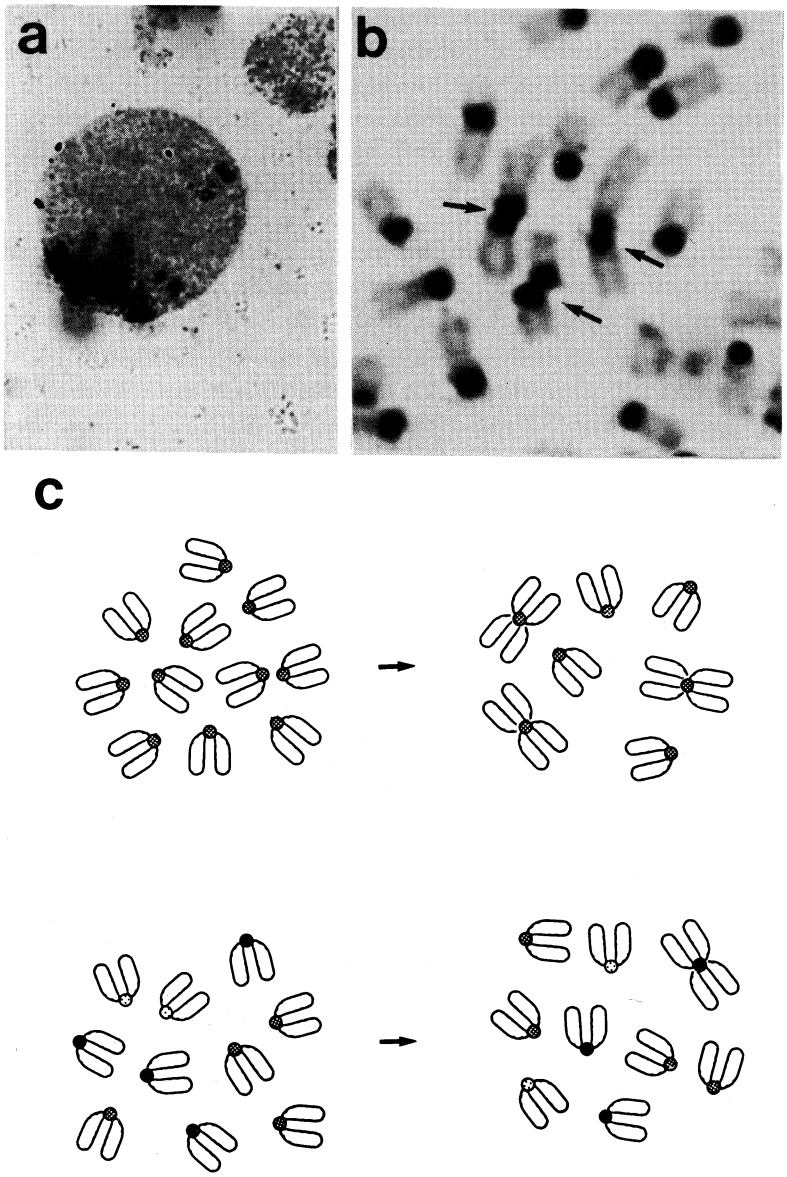

Fig. 2. – Clustering of satellite DNA as revealed by an *in situ* hybridization of sat-DNA probe, both in interphase nuclei (*a*) and in metaphase plates (*b*) of *Mus domesticus* blood culture. The sites of hybridization were detected using an indirect immunoperoxidase procedure amplified by gold and silver. (*c*) In karyotypes with highly repetitive and homogeneous DNA sequences at the pericentromeric areas Rb chromosome formation will be more favoured then in karyotypes with heterogeneous sequences.

sequence on a 3′ parental strand of another chromosome to give a DNA double helix with the canonic (*B*) structure reconstituted, if the polar co-ordinates of one of the two chromosomes were anti-(trans-) rotated over the geometrical plane of which they lie and not over the chromosomal symmetry axis (*i.e.*, the two centromeres face each other, one of the two being turned 180° from an identical original partner position) (fig. 3). In this way a hydroxyl group on the 5′ parental strand meets another group on the 3′ parental strand of the other chromosome, which now has a 5′ pattern of polar co-ordinates, in the proper angular position for joining the two DNA backbones through the formation of a four-stranded structure. The rearranged DNA region would then be cut off by a topoisomerase (nicking-closing enzyme).

The associated loss of base pairs can be extremely small, leading either to the maintenance of both kinetochores or, better, to the inactivation of one of them [14], the mechanism of which could be a frame shift for reading the DNA sequences that codify for one kinetochore), since Rattner and Lin [10] found only one functional kinetochore in mouse Rb chromosomes, with the centric heterochromatin in that area much more condensed.

In this model, no *Z* DNA, strand isomerization, transitional conformation states, or unrolling and rotatory diffusion of the entire DNA molecule need be assumed. A direct prediction of this model is that the mouse Rb chromosomes must show contralateral asymmetry between the thymidine-rich strands (since acrocentrics exhibit lateral asymmetry) with the proper centric orientation of the satellite DNA: effectively they do so [15]. Moreover, since house mouse acrocentrics share all similar satellite DNA sequences, chromosomal arm involvement in Rb metacentrics should be at random: effectively this is the situation, the sex chromosomes and the pair no. 19 being the only exceptions [2, 3, 16].

The mechanism of Rb formation would thus be based on the chemical-physical properties of the DNA itself (*i.e.* base-pairing), and on stochastic events (*i.e.* random favourable spatial relationships of DNA sequences in the pericentromeric regions of two chromosomes), and related to inherent genomic traits, *i.e. high degree of satellite DNA sequence homology*.

If the molecular basis for Rb chromosome formation is base pairing between homologous DNA sequences on two different chromosomes and pertaining to 5′ and 3′ parental strands, one can predict that chromosomes will more easily exchange at sites with greater homology. It is interesting to stress that in humans Tsujimoto *et al.* [17] found identical DNA sequences at the sites where chromosomes 14 and 18 rearrange to give a *t*(14; 18) translocation. Chromosomes 13, 14, 15, 21 and 22 would be good candidates for whole arm translocations, but each of them has sets of satellite fractions different in quantity and quality, and one finds fewer translocations among human chromosomal rearrangements that involve them than would be expected on the basis of random chance for rearrangements [18].

In addition, when satellite DNA constitution and karyotype diversification are compared in some old world monkeys, it emerges that the satellite DNA of a subgroup of three guenons that has $2n$ values varying from 58 to 62, *i.e. Cercopithecus neglectus* ($2n = 58$-62), *C. diana* ($2n = 58$-60) and *C. pygerithrus* ($2n = 60$), is more heterogeneous than that carried by another subgroup of three specie that has $2n$ values varying

from 54 to 72, *i.e. Cercopithecus albogularis* ($2n = 72$), *C. cephus* ($2n = 66$) and *C. (Erythrocebus) patas* ($2n = 54$) [19]. The same is true for cattle, in which the pericentromeric satellite is quite heterogeneous [20], and, in spite of the 60 acrocentric chromosome, Rb chromosomes occur very rarely [21]. Apparent exceptions to the striking relationship between homology of satellite DNA sequence and Rb chromosome formation are human chromosomes 13 and 21: they are not preferentially involved in Robertsonians, even though they carry the same α-satellite.

Moreover, the homologous DNA sequences needed for DNA-DNA hybridization need not necessarily be in the satellite fraction. Transposable elements, or retroviral

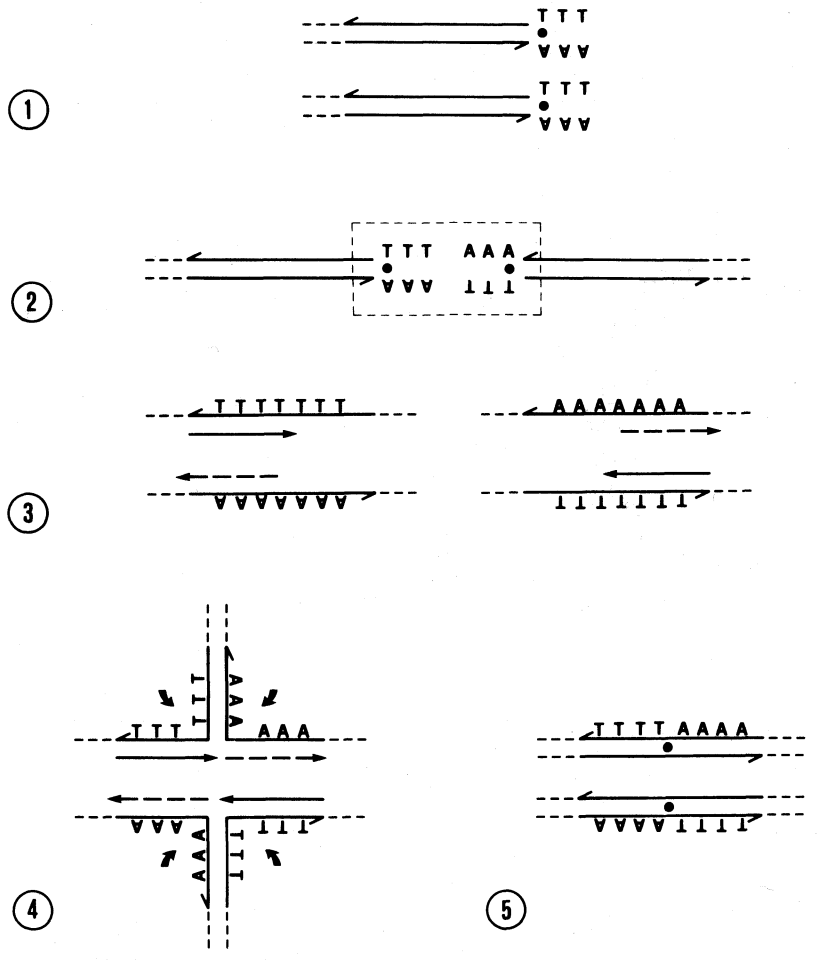

Fig. 3. – Our hypothesis for Robertsonian exchange leading to whole chromosomal arm fusion. If two chromosomes face each other in anti-(trans-)position (*i.e.* one of the two is rotated 180° over the geometrical plane (1 and 2), base pairing could occasionally occurr between homologous DNA sequences on two different chromosomes and pertaining to 5′ and 3′ parental strands (3 and 4). The DNA heteroduplex is cut off by a topoisomerase (arrows) and DNA strands are rejoined by DNA ligase (5). Depending on the location inside the rectangular where the «hybridization» event occurs, the Rb chromosome would maintained either both centromeres, or only one of them; when two centromeres are present, one can be functionally inactivate.

In the resulting Rb chromosome, the pericentromeric heterochromatin maintains its polarity.

genomes incorporated at the telomeric ends (in the mouse roughly 700 000 base pairs on each chromosome are copies of retroviral genomes [22]), can work as well, providing homologous DNA sequences to different chromosomes.

Two last considerations follow from our model, in order to complete the evaluation of the role of Rb fusion in speciation:

i) Rb chromosomes with new arm composition could arise through chromosomal arm exchanges between *pre-existing* Rb chromosomes when chromosomes have similar satellite sequences;

ii) Rb chromosomes could arise in premeiotic cells, *i.e.* spermatogonia or oogonia, since the mechanism hypothesized operates during DNA synthesis.

If one or both of these last mechanisms are active, a rapid karyotypic diversification can occur. Thus, for the house mouse, this reconciles the data regarding a recent origin of the Rb variants, evaluated in Italy on archeological basis no more than 10 000 years [23] and, more generally, on the basis of mitochondrial DNA analysis [24] about 20 000 - 40 000 years. This would greatly simplify Capanna's explanation (successive appearance of new Rb's and hybridization of mouse populations with different sets of Rb's [23]), or Dover's hypothesis (accumulation of Rb metacentrics via purely stochastic phenomena of molecular drive [25]), for the existence of such a large number of populations with Rb chromosomes of different arm composition.

Finally, the role played in our hypothesis by the repetitive DNA sequences, located in the pericentrometric area is consistent with the view that they oversee the karyotypic transformation (*).

(*) The authors thank several colleagues and friends (K. Benirschke, G. Bernardi, G. Corneo, M. Fraccaro, T. C. Hsu, S. Ohno, M. van der Ploeg, P. Polani) whose helpful suggestions are included in the paper. This research has been supported by grants of the Wilkinson Foundation (Charitable Trust), London (C.A.R. and S.G.) and of the Italian National Research Council (E.C.).

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