# Telomere signals in Robertsonian fusion and fission chromosomes: implications for the origin of pseudoaneuploidy

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Running title: Telomere signals in Robertsonian chromosomes

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**Abstract**

In situ hybridization with synthetic plant telomere sequences resulted in labelling of all broad bean (Vicia faba) chromosomes exclusively at terminal positions. Telocentric chromosomes derived by fission of the metacentric satellite chromosome of V. faba also showed signals at both ends while the ancestral metacentric did not reveal signals at its primary constriction, the point of fission. Correspondingly, all acrocentric mouse chromosomes were labelled by in situ hybridization with a vertebrate telomeric probe at both ends of each chromatid exclusively. However, different metacentric Robertsonian chromosomes derived by fusion of defined acrocentrics did not show signals at their primary constrictions.

It is discussed that the mechanism of Robertsonian rearrangements leading to pseudoaneuploid increase or decrease of chromosome numbers cannot be a simple fission or fusion of chromosomes without gain or loss of chromatin material. The additional assumption of deletion of telomeric sequences below a detectable level after fusion and amplification of these sequences following fission is necessary to explain the present observations.

Evolutionary changes of karyotypes may be quantitative (enlargement or reduction of genome size) and/or qualitative (structural rearrangements) and involve either whole chromosome sets, single chromosomes or parts of chromosomes. For a recent review see Schubert et al. (1991).

A special route of evolutionary changes of chromosome complements are alterations of diploid chromosome numbers which preserve the number of long chromosome arms. This results in karyotypes called pseudoaneuploid (Rieger, 1963), and is frequently observed in rodents and primates (White, 1973), but also occurs in other groups of animals as well as in plants (Schubert and Rieger, 1985).

There are various possibilities for pseudoaneuploid increase or decrease of diploid chromosome numbers. The most common variant is called Robertsonian rearrangement, i.e., the combination of two acro- or telocentrics into a metacentric chromosome or, vice versa, fission of a metacentric into two telocentric chromosomes. It is not clear up to now, whether these changes result from reversible fusions and fissions without any gain or loss of chromatin as supposed by Holmquist and Dancis (1980) or from reciprocal translocations (White, 1973). Reciprocal translocations implicate irreversible loss of the small centric fragments in case of fusions and the necessity of donors of a centromere and two telomeres in case of fissions

donors of a centromere and two telomeres in case of fissions (Muller, 1938).

In order to decide between these alternatives we hybridized in situ telomeric sequences to pseudoaneuploid chromosome complements of plants and animals with chromosome numbers increased or decreased due to recent Robertsonian rearrangements. Metacentrics which result from fusion of acro-/telocentrics or become dissociated into telocentrics should show hybridization signals at centromeric positions when reversible fusions or fissions are responsible for Robertsonian rearrangements. These signals should be of higher intensity than those observed at centric ends of the corresponding telocentric chromosomes. This is not to be expected when Robertsonian rearrangements arise by reciprocal translocations. The results obtained exclude mere fusions or fissions as the mechanism of Robertsonian rearrangements in broad bean and mouse.

## Material and Methods

### Mouse

Two mouse stocks with de novo Robertsonian translocations were maintained in homozygous condition: Rb(X.2) (Adler et al., 1989) and Rb(10.14), and one stock was maintained in heterozygous condition: Rb(3.9). All three Robertsonian fusions occurred spontaneously and were

observed in progeny of reciprocal translocation carriers on a (102/ElxC3H/El)$F_1$ background. Air-dried chromosome preparations from bone marrow cells were obtained for each of the Robertsonian translocations by a slight variation of the common procedure (Adler, 1984). The modification consisted of spreading cells on clean dry slides, an additional step of fixation (ethanol/acetic acid 3:1) on the slides, and blow-drying. Slides were stored air tight and refrigerated up to 8 weeks.

Broad bean

Squash preparations were made from root tip meristems of an inbred line "ACB" characterized by a homozygous, multiply reconstructed karyotype with individually distinguishable chromosome pairs (Döbel et al., 1978), and of the self progeny from an individual with a homozygous centric fission transforming the arms of the original metacentric satellite chromosome pair into independent telocentric chromosomes (Schubert and Rieger, 1990).

After immersion in colchicine (2h, 0.05%) and fixation in ethanol: acetic acid (3:1) root tips were digested at 37°C in cellulase (1.5h, 1%) and pectinase (2h, 2%). Both enzymes (Serva) were diluted in sodium citrate buffer (0.1M, pH 4.5-4.8). After squashing, in 45% acetic acid, according to the dry ice method, slides were washed in 96% ethanol,

air dried, and stored in glycerol.

Probes, hybridization procedure, and signal detection

Heptameres of animal (5'-TAACCC-3', see Moyzis et al., 1988) and plant (5'-TAAACCC-3', see Richards and Ausubel, 1988) telomeric repeats were synthesized in sense and antisense orientation and end-labelled with Bio-11-dUTP by terminal deoxynucleotidyltransferase (Gibco/BRL).

Slides and probes were denatured for 10 minutes at 73°C in 70% formamide/2xSSC. Hybridization according to Pinkel et al. (1986) was performed for 15h at 37°C in 50% formamide/2xSSC/10% dextranesulfate with approximately 12 ng probe (sense + antisense) per slide and E. coli tRNA (500 ug/ml) as carrier under coverslips sealed by rubber cement.

Washing: 3 times for 10 minutes in 30% formamide/2xSSC (mouse) or 50% formamide/2xSSC (plant chromosomes) at 40°C, once for 5 minutes in 2xSSC, pH 7, and once for 5 minutes in PN buffer (0.1M $Na_2HPO_4$/ 0.1M $NaH_2PO_4$, pH8, plus 0.1% nonidet P40) at room temperature.

Detection of signals was by means of avidin- or streptavidin-FITC conjugate, and counterstaining with propidium iodide after up to three rounds of amplification by biotinylated antiavidin or antistreptavidin antibody from goat (Vector Laboratories) using a Zeiss Axiophot fluorescence microscope. For photographs, Kodak Ektachrom

P800/1600 films were used, with exposure times of 60 and 90 sec and developed for 3200 ASA.

## Results

### Mouse

The acrocentric mouse chromosomes showed hybridization signals exclusively at both ends of their chromatids. This agrees with the data of Meyne et al (1990) who reported a "telomere only" labelling pattern for the mouse. No interstitial telomeric sequences were detectable. Signals at centric ends were usually as intensive as those at the opposite chromosome ends (Figure 1). Within a given cell all telomeric signals could be detected by changing the focus. No telomere sequences were found at the primary constrictions of three Robertsonian metacentrics (Figure 1a-c, Table 1).

### Broad bean

All chromosomes of the broad bean showed clear hybridization signals exclusively at both ends of each chromatid when hybridized with sense plus antisense oligonucleotides corresponding to the telomere sequences of Arabidopsis thaliana (Richards and Ausubel, 1988). This was true also for an inbred line conaining seven pairs

(4 telocentrics + 10 acrocentrics) instead of the normal six pairs (2 metacentrics + 10 acrocentrics) of chromosomes. The smallest, sometimes not even resolvable, signal occurred at the centric end of the telocentric chromosome pair derived from the satellite arm of the standard metacentric chromosome of V. faba (Figure 2a).

Chromosomes of the multiply reconstructed karyotype of the inbred line "ACB" showed the same "telomere only" labelling pattern. Contrary to the centric ends of the telocentrics, which in the line with seven chromosome pairs represent the separated arms of the original metacentric satellite chromosome, the non-dissociated metacentric of karyotype "ACB" did not exhibit hybridization signals within its centromere (Figure 2b, Table 1).

When vertebrate telomere sequences (Moyzis et al., 1988) were hybridized to plant chromosomes at the same stringency no signals were obtained. Obviously, the different frame of the basic repeats prohibited efficient hybridization.

**Discussion**

If Robertsonian rearrangements result in pseudoaneuploid increase or decrease of chromosome numbers simply via fission of metacentrics or via fusion of acro- or telocentrics, respectively, the following prerequisites have to be accepted:

1) All chromatin material must be preserved during the process of Robertsonian changes to guarantee its reversibility.

2) If acrocentrics with recognizable short arms fuse, one of the centromeres should become inactivated to avoid mitotic/meiotic instability which is typical for dicentric chromosomes.

3) If true telocentrics with short arms consisting just of telomeric sequences fuse, the resulting metacentric possesses a bipartite centromere structure with the two parts separated by telomeric sequences of both original telocentrics.

4) Fission into stable telocentrics is restricted to such metacentric chromosomes which themselves originated by fusion of telocentrics.

The last point is not in accord with the hypothesis of Imai et al. (1986), which considers fusions to be only rare "back eddies" within the general tendency for centric fission in karyotype evolution. On the other hand, it is in keeping with Morescalchi's hypothesis (1990), based on amphibian data, according to which the general tendency of evolution is fusion of chromosomes only occasionally followed by fissions. Since telomeric sequences of vertebrates (Moyzis et al., 1988) and of plants (Richards and Ausubel, 1988) have been characterized the above

material.

The finding of strong hybridization signals with telomeric probes around centromeres of some (but not all) metacentric chromosomes of the Chinese hamster and the rat (Moyzis et al., 1988) and of some other animal species (Meyne et al., 1990) seemed to support the fusion/fission hypothesis of Holmquist and Dancis (1980). The same is true for the observation of a spontaneously fused chromosome in tissue cultures of Sigmodon mascotensis, which apparently has retained telomeric sequences within its pericentric region (Meyne et al., 1990). The occasional occurrence of such signals at interstitial positions (Meyne et al., 1990) could be taken as evidence for possible end-to-end fusions at points distant from centromere(s), and for subsequent inactivation or loss of one of the centromeres; it could also explain the sudden terminal stabilization of open breaks resulting from McClintock's breakage-fusion-bridge cycles. Such breakage-fusion-bridge cycles involving dicentrics at postmeiotic stages were assumed to be responsible for pseudoaneuploid changes of chromosome numbers after irradiation of pollen of Tradescantia (Östergren and Östergren, 1983).

The data presented in this paper show directly the location of telomeric sequences, described by Richards and Ausubel (1988) for Arabidopsis thaliana, at terminal

positions of plant chromosomes. It could be demonstrated that the broad bean belongs to the group of species with a "telomere only" hybridization pattern.

The absense of hybridization signals at centromeric positions of all tested Robertsonian metacentrics of the mouse and of the original metacentric of the broad bean, which recently became separated exactly between two small Giemsa bands inside its primary constriction (Schubert and Rieger, 1990), does not support the fusion/fission hypothesis in its simplest form, which excludes any loss or gain of chromatin. The absence of interstitial telomere signals in autosomes of the Indian muntjak (Scherthan, 1990), which have originated by tandem fusions (Elder and Hsu, 1988), and the absence of signals at centromeres of chromosomes 14 and 18 of the black rat, Rattus rattus (MEYNE et al.1990), which led to fission chromosomes (Yosida et al., 1979), point in the same direction. However, the results presented here do not absolutely rule out the fusion/fission hypothesis, if it is additionally assumed that, during or after fusion, deletions of telomeric repeats occur down to a remnant not resolvable by in situ hybridization but still sufficient to become amplified to provide functional telomere structures immediately after fission.

The alternative translocation hypothesis has to explain the loss of the small centric translocation product arising in the case of symmetric reciprocal translocations as well as the source of one additional centromere plus two telomeres in the case of fission of metacentrics. Explanations of both phenomena meet difficulties. Involvement of centromere-like elements as donors of centromere and telomeres, possibly resulting from large interstitial deletions within extrachromosomes (polysomes or B chromosomes), was proposed for the fly Megaselia scalaris on the basis of electron microscopic data (Wolf et al., 1988).

A molecular analysis of four mono- or dicentric human Robertsonian metacentrics revealed translocation breakpoints at different positions within the short or proximal long arms of the original acrocentrics (Cheung et al., 1990). At least these metacentrics were caused by reciprocal symmetric and asymmetric translocations, respectively. This indicates that possibly both, reciprocal translocations and, alternatively, fusions/fissions may result in Robertsonian interchanges.

Two tests might be needed in individual cases of Robertsonian interchanges to distinguish between an origin via modified fusion/fission and one via translocation mechanisms:

1) A molecular one, i.e., cloning and complete sequencing of centromeric DNA of Robertsonian metacentrics and/or such metacentrics that may become dissociated.

2) A genetic one, i.e., looking for direct reversibility of Robertsonian rearrangements within corresponding progeny.

For methodical reasons the molecular test has not been possible to date. To our knowledge, genetic evidence for direct reversion of Robertsonian fusion is lacking as yet. An experiment to test the reversibility of fissions was recently started with the broad bean inbred line containg two pairs of telocentrics instead of the original pair of metacentric chromosomes.

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**Legends**

**Fig.1** In situ hybridization patterns of mouse chromosomes with the biotinylated telomeric probe. a) metaphase of Rb(X.2), b) metaphase of Rb(10.14), c) metaphase of heterozygous Rb(3.9). Inserts show the G-banded Robertsonian metacentrics. Note the absence of signals at the centromeres of the metacentrics.

**Fig.2** "Telomere only" labelling pattern of of Vicia faba chromosomes after in situ hybridization with the biotinylated telomeric probe. a) complete metaphase of an individual with 2n=14 chromosomes. Both arms of the ancestral metacentric satellite chromosome pair occur as independent telocentrics (arrow heads). The satellite arms can be recognized by their secondary constriction, the long arms form the longest chromosome pair. b) a sample of four metacentric chromosomes of the broad bean karyotype ACB without signals at their unsplit centromere.

**Table 1**

Relations of telomere labelling and labelling of centromeres of Robertsonian (mouse) and unsplit (broad bean) metacentrics

| Robertsonian translocations | Numbers of cells with | |
|---|---|---|
| | complete telomeric signals | centric signals in metacentrics |
| mouse | | |
| Rb(X.2)(X.2) | 20 | 0 |
| Rb(3.9)3,9,Y | 23 | 0 |
| Rb(10.14)(10.14)Y | 23 | 0 |
| Broad bean karyotype ACB: | 15 | 0 |

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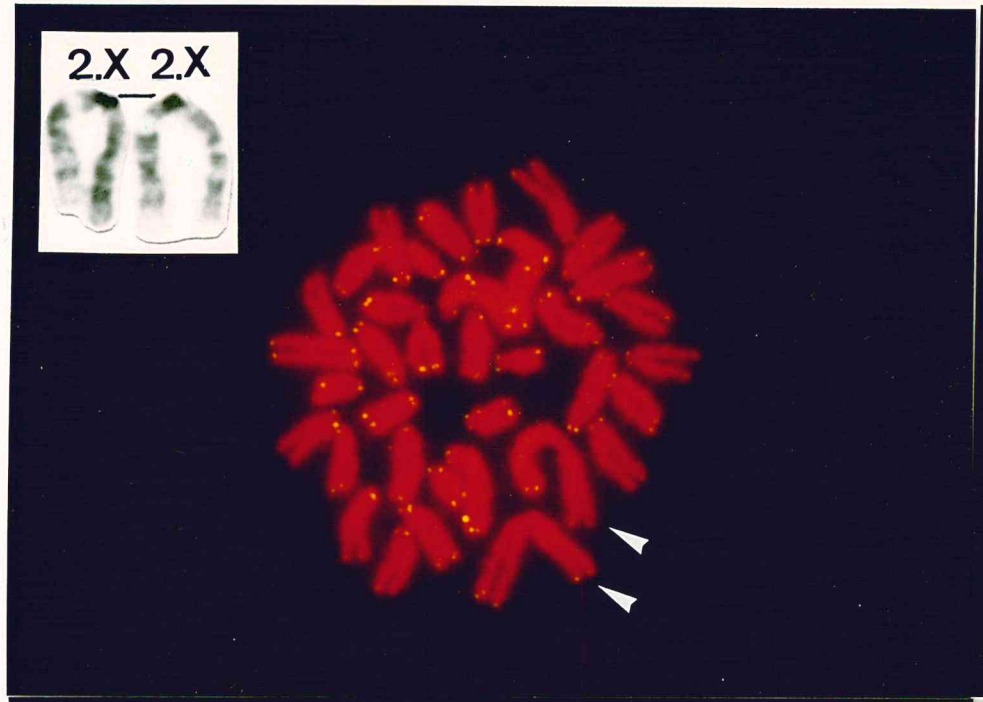
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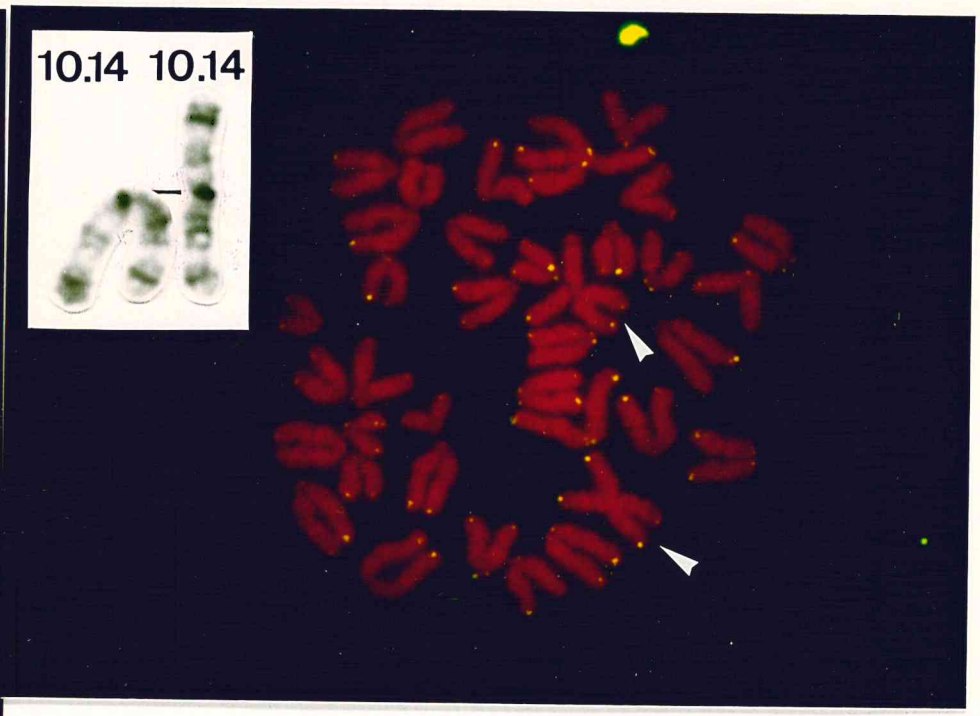
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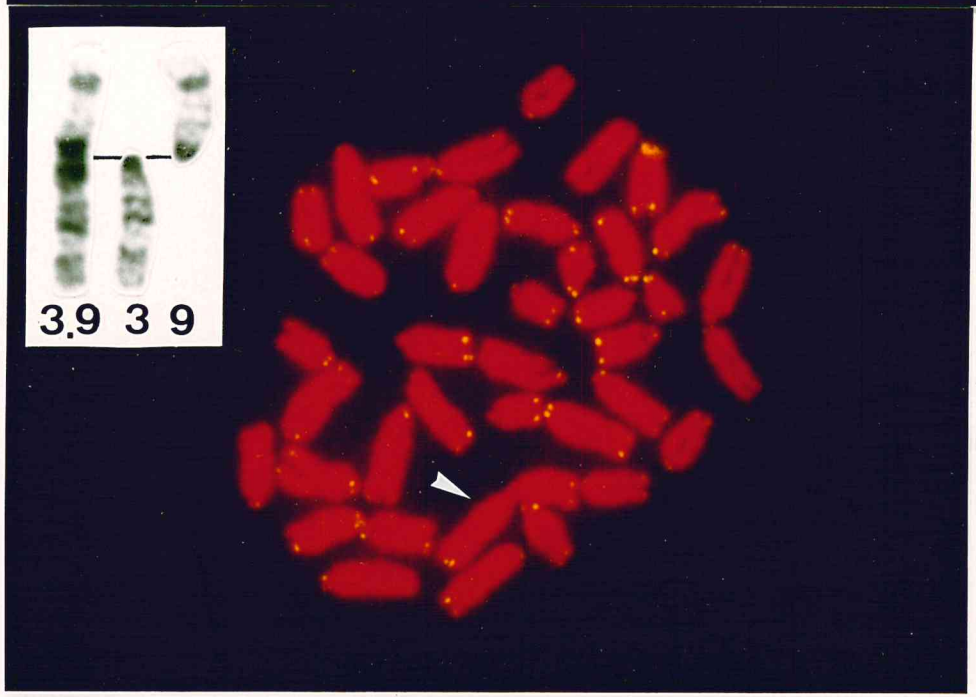
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