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Telomere replication, kinetochore organizers, and satellite DNA evolution

(centromere/Robertsonian rearrangement)

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ABSTRACT **Robertsonian rearrangements demonstrate one-break chromosome rearrangement and the reversible appearance and disappearance of telomeres and centromeres. Such events are quite discordant with classical cytogenetic theories, which assume all chromosome rearrangements to require at least two breaks and consider centromeres and telomeres as immutable structures rather than structures determined by mutable DNA sequences. Cytogenetic data from spontaneous and induced telomere–telomere fusions in mammals support a molecular model of terminal DNA synthesis in which all telomeres are similar and recombine before replication and subsequent separation. This, along with evidence for a hypothetical DNA sequence, the kinetochore organizer, readily explains latent telomeres, latent centromeres, and reversible (one-break) Robertsonian rearrangements. A second model, involving simply recombination between like satellite DNA sequences on different chromosomes, explains not only how one satellite can simultaneously evolve on different chromosomes, but also why satellite DNA is usually located near centromeres or telomeres and why it maintains a preferred orientation with respect to the centromere.**

Robertsonian rearrangement, telomeres, and centromeres

The most easily observed features of a chromosome are its ends (telomeres) and its primary constriction (centromere). Broken chromosome ends, as caused by x-rays or stretching on the spindle, show a capacity for fusion; they behave as if sticky (1) in that they fuse with themselves but not with natural ends (telomeres). McClintock (2) showed that after chromosomes with two broken ends fused, resulting dicentrics were unstable; the two centromeres would often separate to opposite spindle poles and break the chromatid. Muller (1) then formulated two rules of chromosome structure and mechanics. (*i*) All viable chromosome rearrangements require at least two breaks with subsequent rejoining of the broken ends. (*ii*) Rearranged chromosomes must have exactly two telomeres (organelles located at the ends) and one centromere (an internal organelle) to be mechanically stable.

Robertsonian rearrangements between rod chromosomes (Fig. 1 *upper*) to produce metacentric biarmed chromosomes (Fig. 1 *lower*) are a common mechanism of karyotype evolution and occur spontaneously at an appreciable frequency in mammalian tissue culture (5) or even in the somatic tissue of certain fish (6). Reciprocal translocations (Fig. 1*a*) are consistent with Muller's rules. The reverse exchanges, Robertsonian fission of a metacentric into two rod chromosomes, have been observed, and some (Fig. 1 *b* and *c*) appear as one-break rearrangements which do not require a centric fragment to supply a new centromere and telomeres to the new chromosomes (5, 7, 8). In addition, Robertsonian metacentrics generally possess twice the centric structure of rod chromosomes (7–10). In the grasshopper *Neopodismopsis*, Moens' (11) electron micrographs showed this doubled "knob"-like structure to be penetrated by twice as many microtubules as the single centric knob of rod chromosomes. Thus, a metacentric's centric region often appears doubled and capable of splitting by fission, each half becoming a functional centromere (8, 10).

Robertsonian rearrangements, especially the fissions, reveal the inadequacy of Muller's rules, especially his concept of centromeres and telomeres as immutable structures (1), and imply some or all of the following: (*i*) dicentrics can be stable; (*ii*) fissions can result from one-break rearrangements; (*iii*) centromeres and telomeres can reversibly appear and disappear; and (*iv*) centromeres and telomeres can be terminal coincident structures.

Dicentrics can be stable, showing parallel chromatid separation when the two centromeres are close together. Hair (12) observed an isodicentric through many vegetative generations in the plant *Agropyron*. The original dicentric was unstable at mitosis; criss-cross and interlocking separation produced a breakage–fusion cycle that resulted in shorter intercentric distances. Dicentrics with short intercentric regions, however, were mitotically stable, both centromeres on one chromatid separating to the same pole. Dicentrics can also be stable when one centromere is latent (see review, ref. 13). In humans, most Robertsonian metacentrics are dicentric (14) in that they show pericentric heterochromatin from both parental chromosomes; however, one of the centromeres is often inactive in that it does not produce a secondary constriction and region of tight sister-chromatid pairing (15). In one t(7:15)(p21;p11), the C-banding pericentric heterochromatin of chromosome 15 identified the second centric region, but this centromere exhibited neither a primary constriction nor tight sister-chromatid pairing and did not Cd band (16). One interpretation of these data is that a four-break rearrangement occurred, producing a translocation with simultaneous deletion of a centromere. In accord with Hsu *et al.* (13), we alternatively interpret this as a two-break rearrangement involving a translocation and inactivation of a centromere with concomitant loss of the latent centromere's Cd bandedness. Telomeres can also be latent (reviewed in ref. 13), as classically demonstrated during chromatin diminution in *Parascaris* (17). Here, a few polycentric germ-line chromosomes break down into a multitude of small monocentric chromosomes with the *de novo* creation of many telomeres, presumably by activation of preexisting latent telomeres. Activation of a latent telomere appears as a one-break rearrangement that generates two nonsticky ends, telomeres. If a latent telomere were located within a centric region (Fig. 1*b*), activation would also generate a second centromere.

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Abbreviation: KO, kinetochore organizer.

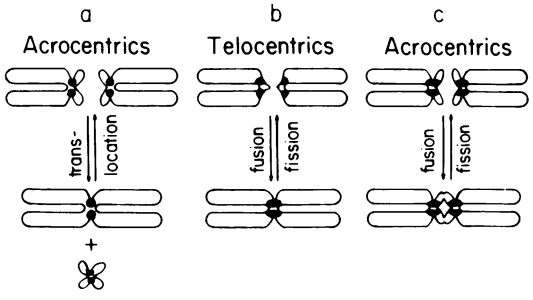

FIG. 1. One translocation and two fission–fusion models of Robertsonian rearrangement are shown with Cd bands (3) produced by active centromeres. (*a*) Reciprocal translocation between acrocentrics produces a metacentric and a small centric fragment which, if subsequently lost, makes this process irreversible. Centromere and telomere number is conserved. (*b*) Reversible fusion of two telocentrics (chromosomes with terminal centromeres) to produce a metacentric. The fusion–fission rearrangement requires only one chromosome break. Two telomeres and a centromere reversibly appear or disappear. (*c*) Reversible fusion of two acrocentrics to produce a metadicentric with a pair of Cd bands (4). Two telomeres reversibly appear or disappear.

The centromere and kinetochore organizer

The active localized centromere of mitotic biarmed chromosomes appears, by light microscopy, as a negatively heteropycnotic constriction (primary constriction) that moves first to the spindle poles during anaphase and is necessary for disjunction of sister chromatids. This region stains by the Cd-banding technique (3) and morphologically is a DNA-containing, bipartite, reverse-repeat structure of several chromomeres flanked by regions of tight sister-chromatid pairing (18). In formalin-fixed electron microscopic sections of both plants and animals, the centromere appears not so much as a constriction, but as a region of lightly staining, thin chromatin fibers (19) in which each sister chromatid possesses a kinetochore plate (20) [pair of kinetochore filaments (19)]. Spindle microtubules penetrate the plate (20) and the plate itself is capable of catalyzing tubulin polymerization (21, 22).

The kinetochore plate is the site of spindle attachment and microtubule polymerization and reflects centromeric activity. It probably also induces the ancillary attributes of an active centromere, including primary constriction, pericentric regions of tight sister-chromatid pairing, and Cd banding. Brinkley and Stubblefield (19) proposed the kinetochore to be a specialized gene, much like the nucleolus organizer defined by McClintock (23), because it contains DNA (18), segregates with the chromatids, and is activated at a specific (mitosis) stage of the cell cycle. We shall call this gene a kinetochore organizer (KO). Just as the nucleolus organizer can be separated from the structural 18S and 25S rRNA genes whose product it organizes (24), the KO need not contain the genes coding for kinetochore-plate proteins, but the KO must organize plate proteins into a functional structure when it is activated. We propose only one additional property of the KO to make it consistent with cytological data (ref. 13; Fig. 1): the KO can be permanently inactivated by rearrangement. Thus, a visible centromere reflects an active KO and a latent centromere, an inactive KO. Viable chromosomes must have at least one active KO for proper disjunction of sister chromatids.

Telomere replication

The chromatid contains one double-stranded DNA helix (25), the termini of which are probably included in its two telomeres. DNA termini cannot be replicated by conventional means because digestion of the terminal RNA primer leaves a 5′ gap (Fig. 2*a*) that no known polymerase can fill (26). Solutions to this

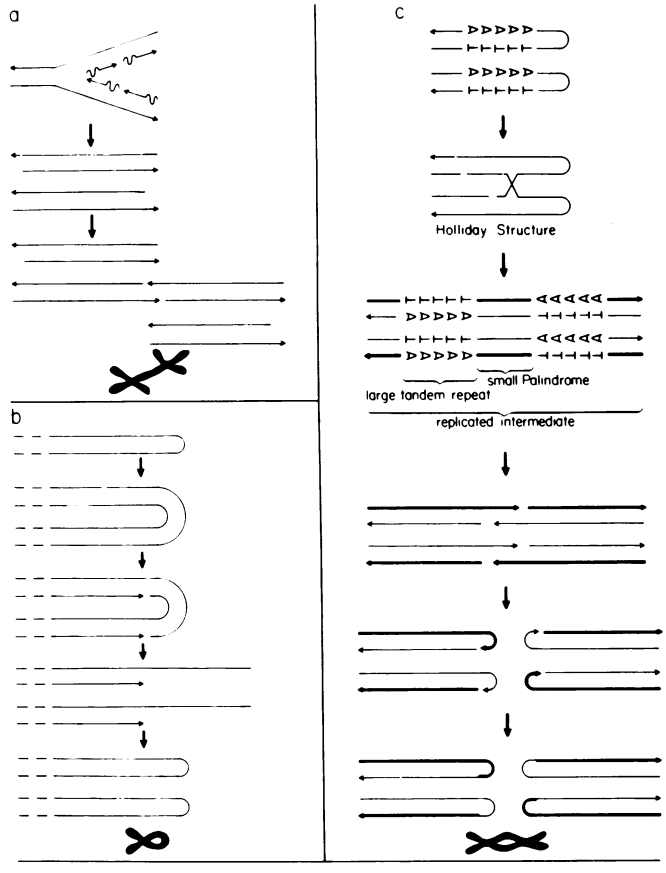

FIG. 2. Types of fused metaphase chromosomes arising as replication intermediates from various models of telomere replication. (*a*) Replication with digestion of RNA primers and ligation of Okasaki fragments leaves terminal 5′ gaps (26). If telomeres are complementary as in T7 phage (26), complementary gaps could base pair, forming concatameric telomere fusions, which, being endless, are able to be replicated. Alternatively, ligation and staggered nicking (not shown) of the fused junction could convert it into a pair of replicable 3′ gaps (27). (*b*) Bateman (28) assumed the telomere to be a covalently closed hairpin, making it different from a "sticky" broken end. Replication generates a palindrome that is nicked by a restriction endonuclease, refolded, and ligated. (*c*) A fusion-before-replication model. All telomeres are identical, covalently closed, and contain a large, repeated sequence, the basic unit of which is represented by the letters A and T. Two telomeres recombine, forming a Holliday (29) structure, which, after ligation, has no ends and is therefore able to be replicated. Newly replicated DNA is represented by the darker lines. Fission of the replicated intermediate proceeds as in *b*.

Okasaki terminal dilemma for eukaryotic telomeres have been proposed (26–28, 30), and representatives are shown (Fig. 2 *a* and *b*). To explain cytogenetic data, we proposed that the telomeres must fuse pairwise before replication (31, 32). The most reasonable molecular model (Fig. 2*c*) required all telomeres in a cell to recombine via common sequences before replication and is consistent with Rubin's (33) cloning of a 12-kilobase DNA sequence, four tandem repeats of a 3-kilobase sequence, which hybridized *in situ* to all *Drosophila melanogaster* telomeres and to the ectopic strands that connect them, and with Forte's and Fangman's (34) discovery that yeast (*Saccharomyces cerevisiae*) telomeres are covalently closed hairpins.

Eukaryotic chromosomes, in some special instances, appear fused end to end, and in rare extreme cases the entire genome appears as a giant ring of fused chromosomes (35–37). White (38) inferred that telomere base-sequence homology caused occasional end-to-end associations of meiotic chromosomes. We extended this concept of homology by interpreting mitotic associations as replication intermediates (31, 32). Senescing

human primary cell lines (39) and lymphocytes from patients with Thiberge–Weissenbach syndrome (40) behave as if the restriction endonuclease of Fig. 2*c* does not cut efficiently in that many chromosomes are seen permanently fused end to end at the first mitoses after fusion. The fused configurations predicted by other replication models (Fig. 2 *a* and *b*) were not seen. Instead, sister telomeres were fused to another pair of sister telomeres (Fig. 2*c*), as is consistent with telomere fusions occurring before replication. Random telomere pairs were fused; all telomeres were similar in their fusion potential (39, 40) and not composed of complementary pairs as in a concatameric fusion process (26) (Fig. 2*a*). BrdUrd pulse labeling indicated that the chromatin at the fusion junction of Thiberge–Weissenbach lymphocyte chromosomes replicated very early in the S phase preceding mitotic fusion (40). This suggests that in normal cells, telomere–telomere fusion is a temporary event occurring in early S phase and would not be detected in asynchronous cultures by either viscometric DNA molecular weight analyses (25) or by psoralin crosslinking (41) to reveal the cloverleaf-shaped Holliday intermediate of Fig. 2*c*. Fig. 3*a*-type telomere–telomere fusion figures were induced in the first metaphase after treatment of mouse cells with mitomycin C, a DNA crosslinking agent (42). Such figures would, however, be expected from the replication model in Fig. 2*c*. If one crosslink prevented terminal branch migration of the Holliday structure, one pair of chromatids would separate after replication while the other pair would remain fused (32). In conclusion, we view telomeres as structures that contain DNA termini and solve the special problem of terminal replication. Cytogenetic data support a model requiring telomere–telomere fusion before replication, and molecular data are most consistent with a recombination (Fig. 2*c*) mechanism of fusion. The model also explains latent telomeres and some aspects of Robertsonian fusion.

Telomeres (stable nonsticky chromosome ends) are created *de novo* during the differentiation of soma from germ line in *Parascaris*. This is easily explained if differentiation promotes the separation of latent telomeres (fused telomere pairs) or, more specifically, activates a restriction endonuclease that cleaves those palindromes (Fig. 2*c*) that are latent telomeres (27). Activation of a latent telomere would appear cytogenetically as a one-break rearrangement, generating two telomeres, and would violate Muller's (1) rules. Robertsonian fusion would be a special case of telomere–telomere fusion, involving the centric ends of two rod chromosomes. In the molecular model (Fig. 2*c*), a mutation in a terminal hairpin sequence could prevent recognition by the restriction endonuclease of the replication intermediate's palindrome (Fig. 2*c*), resulting in fusion. A subsequent back mutation would appear as Robertsonian fission of a latent telomere.

The fusion-first replication mechanism necessitates symmetry. Specifically, if one telomere contained an inverted Rubin sequence, it could fuse with other telomeres but the intermediate could not separate (32). Thus, telomeres with a common Rubin-sequence orientation would be selected for, as portrayed in Fig. 3*c* by the letters A pointing toward the terminal hairpin. The letters A are also pointing away from an internally located centromere; thus we can define for all such telomeres a common centric orientation.

Centric orientation and satellite DNA evolution

Mouse satellite DNA sequences all have the same centric orientation (Fig. 3*a*). Cytologists found this by using bromodeoxyuridine's quenching of fluorescent dyes. They followed the T-rich satellite strands during a few generations of BrdUrd incorporation and determined their relative orientations by

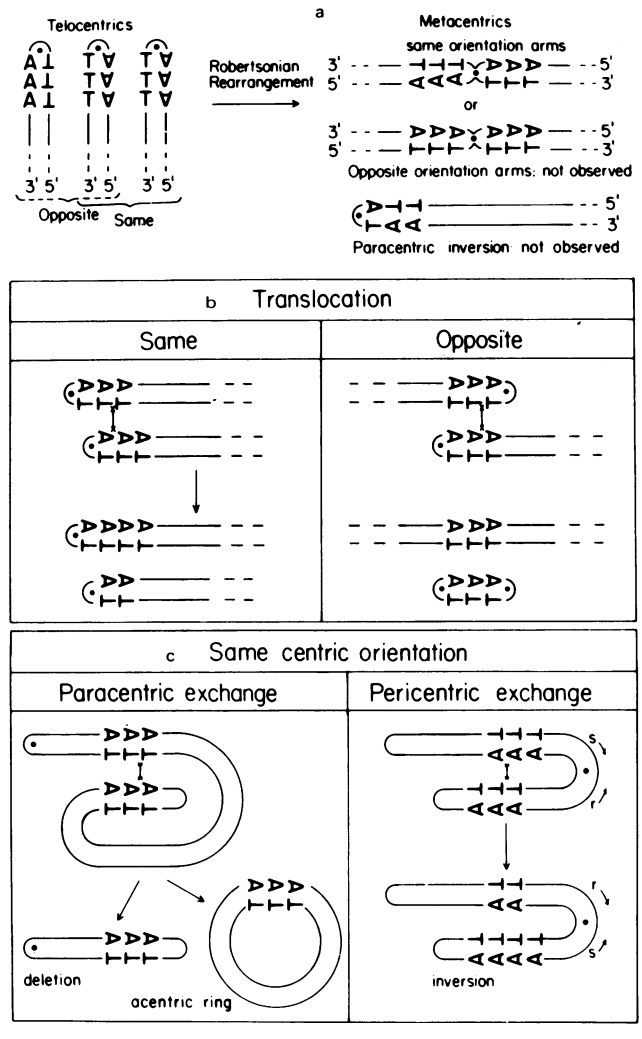

FIG. 3. Centric orientation of mouse satellite DNA around the KO (dot). (*a*) The relative centric orientation of satellite blocks is determined by the segregation of T-rich satellite strands in metacentrics. (*b*) A base-pairing-dependent translocation (X—X) between terminal blocks of either opposite or similarly oriented satellite DNA would produce either an acentric chromosome and dicentric fragment or a terminal exchange. (*c*) The product(s) of intrachromosomal, base-pairing-dependent exchanges between similarly oriented satellites. Centric orientation (arrows) of the included sequences remains unchanged. Because of the base-pairing requirement, the paracentric exchange is a deletion and not an inversion as in *a*.

their segregation in Robertsonian metacentrics (43) and x-ray-induced dicentrics (44). Even in mouse L-cell marker chromosomes, which were generated by multibreak rearrangement and contain many intercalary blocks of satellite, all satellites' centric orientations were invariably maintained the same (45). A paracentric inversion is one of several rearrangements that can reverse (Fig. 3*a*) and thus randomize centric orientation, but evidence for this was not found‡ (45). Selection must act to preserve a common centric orientation, and any tenable theory of satellite DNA evolution must explain this fact.

Computer simulations by Smith (47) have shown that DNA whose sequence is not maintained by selection can theoretically develop a hierarchy of tandem periodicities (i.e., form satellite

‡ When, as in humans, two or more different blocks of A·T-rich satellite DNA are present on the same chromosome arm, switches in T-rich strands (46) may represent the junction of two different satellite blocks and not a switch in any one satellite's centric orientation.

sequences) as a result of base pairing between sister chromatids followed by unequal sister-chromatid exchange at the pairing site. The patterns of restriction-endonuclease-sensitive sites observed in mouse (48), *D. melanogaster* (49), and calf (50) satellite DNA definitely support Smith's prediction of a hierarchial repeat pattern of long-range periodicities. Two consequences of this base-pairing-dependent mechanism are that satellites evolve in blocks, contiguous stretches of like sequences as shown for *Drosophila* (51, 52), and that the sequences within any one block have the same orientation, as shown for mouse satellite (43).

Bovìni and Caprini, of the superfamily Bovidae, diverged over 10^7 years ago from a common ancestor and yet they still share a weakly cross-hybridizing satellite DNA (53). For any one species in the superfamily, the pattern of restriction-endonuclease-sensitive sites of the satellite is a simple hierarchical one (50) even though the satellite DNA comes from many different chromosomes. The simple pattern means that within a species, the satellite on many different chromosomes is very similar in its long-range periodicities. A recent saltatory event with subsequent dispersion to the different chromosomes cannot explain this because the same satellite was presumably present on different chromosomes 10^7 years ago. The satellite must have coevolved on different chromosomes, with satellite mutations being communicated from chromosome to chromosome by some interchromosomal interaction.

Base-pairing-dependent interchromosomal exchange between satellite blocks of like sequence would explain both coevolution and centric orientation of satellites. Such an exchange between terminal satellite blocks on different chromosomes would produce an acentric chromosome if the blocks were of opposite centric orientation (Fig. 3*b*) and result in selection against opposite orientations in one species. Terminal satellites, as in the mouse, could thus coevolve by exchange and maintain a particular orientation relative to the KO because the structure the KO organizes is essential to the existence of each chromatid. Similar exchanges occurring between satellites of the same orientation on the same chromosomes would determine a preferred distribution of satellite blocks. Paracentric exchanges would delete intervening genes (Fig. 3*c*) so that homologous satellite blocks on the same chromosome arm would be selected against. Similarly, pericentric exchanges could alter linkage groups (Fig. 3*c*), and interstitial homologous satellites on opposite arms would be disfavored. However, if the blocks were pericentric or terminal as in most eukaryotes, such exchanges would not alter linkage groups. This postulated exchange mechanism presents a dynamic view of satellite evolution with blocks of similar sequence located usually pericentric or terminal and showing their dynamic state through population polymorphisms of block sizes or pericentric inversions, as consistent with present cytogenetic data.

The whole chromosome

Muller (1) considered centromeres and telomeres as immutable structures; by associating structure with the location of the structural determinant, he thought a telomere and centromere could not share the same location, as in a telocentric, thus obscuring the mechanism of reversible Robertsonian rearrangements (Fig. 1). We define active telomeres as structures (the kinetochore plate and ancillary centric chromomeres) expressing KO DNA sequences. Thus, a telocentric chromosome could have a terminal centromere (kinetochore plate overlapping the telomere) and a telomere that functions for terminal replication without having the KO as a terminal sequence (Fig. 4*b*). Similarly, if fusion of telocentrics brought two KOs close together so that the plates they organized overlapped (Fig. 4*b*),

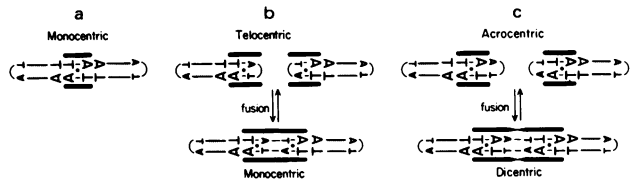

FIG. 4. (*a*) A monocentric chromatid has one kinetochore plate (thick line) which is organized by the physically smaller KO sequence (dot) and contains pericentric satellite (large letters) and telomeric Rubin sequences (small letters). Telomere fusion can be transient, as in replication (Fig. 2*c*), or permanent, as in Robertsonian fission–fusion (*b* and *c*). Satellite and Rubin sequences maintain a constant relative orientation; thus, telomere–telomere fusions produce the contralateral symmetry of pericentric satellite observed in mouse metacentrics (Fig. 3*a*). In *c*, the kinetochore plates do not cover the ends of the acrocentrics and show a doubled nature in the fused metadicentric.

the fusion product would have one centromere, one centromere and two telomeres having disappeared during the fusion. This would also explain why the centromere of Robertsonian metacentrics often shows a doubled nature (7–11). Fig. 4 also indicates that latent telomeres should be common in centric regions and is consistent with the observation of Kato *et al.* (5) that 2.9% of the cells in their CHO line showed evidence of at least one fission event, spontaneous breaking of the centromere with concomitant "healing" of the broken ends.

The molecular models we present, while probably inaccurate in detail, do unite disparate data and, more importantly, remove the mysteriously immutable or discrete structural properties ascribed to telomeres and centromeres, presenting them instead as the expressions of DNA sequences that can mutate, rearrange, and be regulated.

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