PUBLICATIONS
PERSPECTIVES IN MEIOSIS: SYNAPTONEMAL COMPLEX

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ABSTRACT

Synaptonemal complexes (SCs) are ubiquitous, evolutionarily stable protein structures associated with chromosome pairing and recombination during meiosis. This review presents a comprehensive account of structure and function of synaptonemal complexes from a cytogenetical point of view. SC karyotyping enables identification of natural groups of organisms and hybrids.

Key words: synaptonemal complex, pairing, meiosis.

INTRODUCTION STRUCTURE AND DEVELOPMENT OF SC

Synaptonemal complexes (SCs) are zipper-like, protein structures assembled during the first meiotic prophase. Their discovery by Fawcet and Moses independently in 1956 has provided an additional dimension to the study of meiotic chromosome behaviour (Moens 1978). Wahrman (1981) prefers to call them Synapton. These structures are implicated in homologous chromosome pairing in the first meiotic prophase. The ability of the SC to associate the homology search of chromosomes with the physical orientation of kinetochores is the basic process that underlies the laws of Mendel (Hawley and Arbel 1993). This review presents recent information on the significance of SC from a cytogenetical point of view based on findings in a wide range of organisms.

The SCs represent a common theme among eukaryotes (Westergaard and Wettstein 1972). Wettstein et al. (1984) observe that the universal presence of the SC is indicative of its evolutionary stability. SC are reported in all the higher plants examined besides some groups of algae (Krugen and West 1972; Toth and Markey 1973; Braten and Nordby 1973); some ascomycetes (Zickler 1973); and Saccharomyces cereviseae (Zickler and Olsen 1975). The SCs are remarkably similar in architecture in organisms ranging from mammals to fungi (Dresser 1987). Two species, Schizosaccharomyces pombe (Kohli and Bahlke 1994) and Aspergillus nidulans (Egel-Mitapi et al. 1982) display meiosis without a detectable SC.

STRUCTURE AND DEVELOPMENT OF SC

The SC in the fully expressed form occurs throughout the length of the homologous chromosomes (bivalent) as a tripartite, ribbon like structure as seen in pachytene of the first meiotic division (Westergaard and Wettstein 1972; Gillies 1973; Albini 1994). The two sister chromatids of a meiotic prophase chromosome develop a single proteinaceous axis, called the axial element or axial core. With advancement of the prophase, these axial cores are connected along their length by numerous transverse filaments (TF) (Wettstein et al. 1984). Axial cores within the SCs are referred to as lateral elements (LE). Between the two LEs, a central element (CE) is formed on the TFs. Both the LE together with the CE form the tripartite structure of SC. Three to five layers of TFs are found within the CE (Schemekel et al. 1993). Though the tripartite structure is generally conserved, details of structure of CE and appearance of LE vary (Schemekel and Daneholt 1995). Even when dissociated from chromatin fibres, SC retains both the lateral and central elements (Gassner 1969). The components of SC can also self assemble in the absence of chromosomes into SC or poly SCs as in reproductive structure of protists, animals and fungi. Therefore, assembly of SC and SC like structures is considered an inherent property of SC components not requiring the presence of DNA or chromosomes (Moens 1994).

The details of length and constitution of SC are evident under the electron microscope. Reconstruction form serially sectioned nuclei has
helped in the analysis of SCs of all bivalents of a genome (Moens 1973; Sotelo et al. 1973; Gillies 1973; Carpenter 1975). Among the other techniques used to demonstrate and study the SC, salt solution spreading of whole mount meiotic chromosomes shows much better preserved SCs (Counce and Meyer 1973; Gillies 1973; Carpenter 1975). Surface spreading techniques have opened up possibilities of direct observation of SCs (Santos et al. 1995). These techniques have been applied to the study of pairing (Gillies 1985; Albini and Jones 1987), structural rearrangements and changes in chromosome numbers (Albini and Jones 1989). Gillies (1973) has also shown that the total length of the SCs of a haploid genome represents about 0.015% of total DNA length. In Drosophila melanogaster the SCs are about 110 um while the DNA length is about 61 mm, with the lateral component of the SCs covering only about 0.2% of the double helix. In maize, the lateral component is between 0.017 to 0.014% of the total DNA length. Thus, only a small portion of the DNA is covered by the SCs.

The length of the SC is manifested fully at the pachytene stage and is an important parameter. The absolute length of the SCs can vary but their relative length remains the same (Dresser 1987). Carpenter (1975) noted almost halving of SC length between early and mid pachytene stages in Drosophila while Moens (1973) found a 20% condensation of Locusta bivalents resulted in disappearance of SCs. Three dimensional reconstruction of SCs from serial sections of pachytene nuclei helps in the determination of chromosome number and karyotyping of organisms with small chromosomes (Moens and Perkins 1969; Byers and Goestch 1975; Moses et al. 1977; Sherman and Stack 1992). Some groups of organisms are characterised by a typical ultrastructure of their SC (Wahrman 1981). Centromeres can also be identified by SC associated centromere structures (Moses et al 1977). Pachytene SC preparations have proved a good target for fluorescent in situ hybridisation (FISH) with DNA probes both in mammals (Moens and Pearlman 1990) and higher plants (Albini and Schwarzacher 1992). SC and nucleolar morphology can aid in preliminary screening of hybrid genotypes (Jenkins and Jimenez 1995).

The meiotic prophase kinetochore consists of a highly stained ball of fine fibrils (Counce and Meyer 1973) through which the SC passes uninterrupted. Each lateral component organises a single kinetochore, and the two homologous kinetochores fuse to form a single kinetochore of the pachytene bivalent (Counce and Meyer 1973; Gillies 1975). The rate of SC formation in the centromeric regions is retarded (Rasmussen and Holm 1980).

Synaptosomal complexes are relatively short lived organelles related to synopsis-exchange (Wahren 1981). The transition from pachytene to diplotene marks the degradation of the SC (Weststein et al. 1984). There is a debate as to whether SCs persist till disintegration of nuclear envelop and kinetochore-MT association stages (Dickinson 1987). However, Gassner (1969) states that SC dissociates from the chromosomes at Anaphase I. Usually the SCs are attached with their both ends to the nuclear envelop at pachytene, and by midpachytene, SCs have coils along their entire length (Moens and Perkins 1969; Holm and Rasmussen 1977; Zickler 1977). Following crossingover, continued chromosome condensation together with repulsion of homologues leads to breakdown of SCs except in regions where chiasmata are formed.

From biochemical point of view, the SC is a metastable structure which disappears after pachytene except in those cases where it acquires some novel post-meiotic functions (Fiil and Moens 1973). Several proteins have been identified in it (Moens et al 1987; Heyting et al 1988). The main protein components of SC are formed between leptotene to zygote stage of prophase (Gillies 1975). Homology search is by a single stranded DNA coated with specific proteins similar to E. coli. Rec. A protein (West 1992). Hotta et al (1984) have detected such single strands of DNA associated with chromosome pairing in meiotic cells of lilies. Myosin has been identified along the bivalent, probably as a CE component (Wahrman 1981). For the assembly of SC, the proteins of the central region (Zip 1 in yeast, SCP 1 in rats and a comparable SYN in hamsters) have a structure indicative of protein-protein interactions that can
account for SC central region (Meuwissen et al. 1992; Sym et al. 1993). Each lateral element of SC is derived from a pair of condensed sister chromatids and, is called an axial element. Most part of the chromatids is located outside the SC and is folded into loops that are attached to the base of the lateral element (Roeder 1990). The LEs of SC are rich in DNA whereas very little DNA is found in the central region (Vazquez-Nin et al. 1993). Several proteins that are not meiosis specific, have also been reported in association with SC, such as topoisomerase II found localised in the vicinity of SC (Klein et al. 1992; Moens and Earnshaw 1989), RAP1 found at the telomeric ends of first meiotic prophase chromosomes (Klein et al. 1992) and RAD 51, a Rec. A homolog (Bishop 1994; Tarasawa et al. 1992). RNA has also been reported in the SC (Moens 1994).

At the ultrastructural level, each bivalent at pachytene is completely paired and shows a SC throughout its length (Rasmussen 1976; Holm and Rasmussen 1977: Carmi et al. 1978). The SC at this stage attains its most distinct appearance with its two lateral elements flanking the chromatids held in register by the central component. The central component appears amorphous in meiocytes of man and Schizophyllum sp. while it is scalariform in oocytes of Bombyx mori. Wahrman (1981) describes CE as a longitudinal fibre sometimes periodically interrupted or as a system of periodically spaced fibres perpendicular to the lateral elements. The width of the central region is about 100-200 nm in Schizophyllum sp. (Carmi et al. 1978) while in Bombyx mori it is about 70 to 80 nm (Rasmussen 1976).

SC IN CHIASMATE ORGANISMS

The appearance of SCs in the first meiotic prophase is associated with cytogenetic events such as pairing and crossingover. At meiosis in chiasmate organisms, chromosomal disjunction and reductional segregation of sister centromeres are integrated with SC functions as shown by immunocyto logical and in situ hybridisation experiments (Moens and Spyropoulas 1995).

Homologue pairing is generally interpreted as a fucntion of SC formation though in mouse and man, FISH studies have shown that pairing of homologues begins before SC assembles (Scherthan et al. See Heyting 1996). After SC dissolves, homologues remain connected by chiasmata (Jones 1987) which are chromatin bridges between nonsister chromatids (Roeder 1990). The synaptic protein Syn 1, present between the cores of paired homologues chromosomes during pachytene, is lost from SC during separation of homologues at diplotene. This separation is influenced by chiasmata (Moens and Spyropoulas 1995). In diploids, SC normally connects two homologues. Under competitive conditions of pairing as in autopolyploids, more than two chromosomes form a homologous group. In these cases, at any given region, two homologues synapese except in a few organisms, that show multiple SC formation. In triploid Bombyx mori, meiocytes were observed with the two homologues with an SC and the third homologue lying at some distance parallel to the bivalent (Rasmussen 1977). In triploid Allium sphaerocephalum (Loidl and Jones 1986), a network of linked SCs seen at late pachytene is interpreted as an attempt to achieve a highest degree of pairing under a random end pairing model. In Lolium amphidiploids, Jenkins and Jimenez (1995) envisage a correction of multivalent configurations to bivalents by dissolution and reassembly of SCs. This method of bivalent formation is adopted by a variety of allopolyploid organisms (Jenkins and Rees 1991).

In a vast majority of organisms, SC provides a structural frame work ensuring the proximity of the homologues DNA regions of the pairing chromosomes (Rasmussen and Holm 1980). Recognition of homologues may be initiated by nonreciproc al events as envisaged by Meselson and Radding (1975). A single strand detached from one chromatid may extend across the SC and become integrated into a recipient duplex. For pairing of homologous chromosomes, SCs are envisaged as structures which can hold the chromosomes together while the base pairing for crossingover occurs at a few places around their periphery. Alternatively, the SCs may accumulate the meeting of the short segments across their central region (Maguire, 1977). For this purpose, the homologues must at least be segmentally


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SC formation is initiated. Gillies and Hotta (1973) consider that alignment of homologues parts of the pairing chromosomes and formation as two dynamic processes of homologous synapsis. When a critical distance 000 to 3000 A is reached between the homologues, SC formation begins (Moens 1973; ch 1973; Gillies 1975). The formation of SC rates the DNA of the homologous homologous nodule. In the absence of intact DNA, SC formation begins (Moens 1973, ch 1973; Gillies 1975). The formation of SC is initiated by remnants of SC seen with each homologous chromosome (Counce and Meyer 1973; Gillies 1975). A DNA binding protein is contained within the central regions of SC (Counce and Meyer 1973; Gillies 1975). According to Gillies (1975) meiotic crossing over is accompanied by the presence of SC. That SC plays a role in chiasma formation is indicated by remnants of SC seen with mata in some organisms even after bulk of C has dissolved (Wettstein et al. 1984) and t mutants with reduced levels of nagination fail to develop SCs (Engelbrecht 1990; Roeder 1990). SCs are now considered control both the number and distribution of reciprocal exchanges between homologous homologous, nodule (RNs), the units which have been reported in many organisms, at least one per bivalent. It appears that the central region of the SC in particular is required for conditions of cross over interference (Egel 1995). According to zickler et al. (1992) recombination nodules work only in the environment of SCs, not with them. There is apparently a RN-independent pathway of reciprocal recombination that operates specifically at recombinational hotspots (Ashley 1994).

Several authors favour the concept that SC further strengthens the bonds that have formed at the primary homology contact. A continuous SC would make any chromosomal region capable of recombination (Loidl 1990). In the absence of genetic recombination and chiasma formation, homologous chromosomes dissociate at diplotene along with SC dissolution (Roeder 1990). In addition, SCs are implicated in: (a) chiasma maintenance (Maguire 1977, 1984), (b) sister chromatid cohesion (Moens and Spyropoulos 1995), (c) signal for chiasma interference (Maguire 1988), (d) prophase chromosome condensation (Loidl 1990) (e) resolution of interlocking (Wischmann 1986) and (f) organisation of kinetochore in first meiotic prophase (Counce and Meyer 1973; Gillies 1975).

**SC IN ACHIASMATE ORGANISMS**

The function of SC in achiasmate organisms is still uncertain. In asynoptic wheat, the lateral elements of the unpaired chromosomes are perfectly developed but they fail to join into an SC (La Cour 1970). The authors suggest that the central element substance is not synthesised in such cases. However, no explanation is offered in triyloid Allium amplexicans which has normal SC but no chiasmata (Stack 1973). Thus, in consistence in relating SC with meiotic events have cropped up. There are cases of paired chromosomes with SC but no recombination as in the oocytes of Lepidoptera (Rasmussen 1977). Presence of SCs in a number of haploid plant pollen mother cells (tomato, maize, barley) has been noted although SCs are not expected to be present in the absence of pairing partners. Abundant SCs in diploid yeast Saccharomyces cerevisiae with very little homologues indicates that the homology as such is
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not a strict requirement for SC formation (Loidl et al. 1991). In the achiasmate male meiosis of praying mantis, Bolbe nigra, short segments of SC were found in metaphase - I bivalents. It is conceivable that SC has a substitutory role for chiasmata in this system (Wolf 1994). In silk moth Bombyx mori, the females have no crossing over and lack chiasmata at meiosis; yet both males and females show SC (King and Akai 1971; Rasmussen 1976). The oocyte nuclei show localized enlargement of SC lateral elements (130 to 100 nm) which may prevent crossing over taking place (Rasmussen 1976).

SC IN HETEROZYGOUS ORGANISMS

Heterozygosity in organisms beyond a threshold seems to affect the formation of SC. For instance, the clockwise coiling of SCs in boat lily, thoeo discolor, is considered an abnormality in view of the permanent translocation heterozygosity of the plant (Moens 1972; Mcquade and Wells 1975). However, in the inversion heterozygotes of grasshopper Keyacris secura, hiasma formation within the inversion is prevented by non-homologous pairing (Fletcher and Hewitt 1977) while no information is available on the formation of SC. It is in such unusual meiotic systems that further research holds exciting opportunities.

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Meiosis-I in *Rhoeo spatheae* – A Contemporary View

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Summary

This paper discusses different aspects of meiosis-I in the permanent translocation heterozygote, *Rhoeo spatheae* (Commelinaceae) in the context of contemporary information in bivalent forming, chiasmate organisms. Significance of neutral crossovers occurring in the telomeres is also discussed with reference to chromosome segregation and meiosis. The problems and prospects of chromosome segregation in triploid *R. spatheae* are pointed out.

Introduction

*Rhoeo spatheae* (Swartz) Stearn (*R. discolor* L’Hetrtier) is the only species under the genus *Rhoeo* Hance of Commelinaceae. The most striking meiotic characteristic of diploid (2n = 12) is a ring of 12 chromosomes at late prophase-I and metaphase-I (fig. 1). The taxon is interpreted as a complete translocation heterozygote (BELLING, 1927; DARLINGTON, 1929; SAX, 1931; SIMMONDS, 1945). This condition presumably arises due to a series of translocations between nonhomologous chromosomes that results in diploid heterozygotes (STACK and SOULIERE, 1984). The heterozygous condition is probably maintained by balanced lethal genes (SAX, 1931; WIMBER, 1968; ZIMMERMAN, 1968). The structural differences in the chromosomes were sufficient for SAX (1931) to map the ring. The metaphase-I ring chromosomes show arms of almost equal length (NATARAJAN and NATARAJAN, 1982, GARCIA-VELAZQUEZ, 1991). Two arms of each chromosome have respective homologous portions on arms of two other chromosomes (BELLING, 1927). Thus, the position of each chromosome is fixed in the ring (LIN and PADDOCK, 1973a).
In principle, chromosomes of maternal and paternal sets move to different poles at meiosis-I and there is no choice in this (SYBENGA, 1975). In *R. spathacea*, only an alternate segregation of chromosomes from the ring results in viable spores (LIN and Paddock, 1978). Each viable spore has six chromosomes, forming either an alpha or a beta complex and the diploid (2n = 12) has both the sets (LIN and Paddock, 1978). Bivalent forming diplonds (n = 6) (Wimber, 1968) though very rare, indicate that the present situation of ring formation is a derived condition. Consequently, success in meiosis-I depends on suitable modifications in the cell cycle checkpoints. A high percentage of pollen viability indicates that such modifications have indeed occurred. The second meiosis is equational and conforms to the cytological events of those in the bivalent forming higher plants.

Most cells possess checkpoints that monitor the integrity of the spindle as well as its attachment to the chromosomes (Yen and Schaar, 1996). In both mitosis and meiosis, metaphase checkpoints are essential to inhibit entry into anaphase until certain specific criteria are met (Murray, 1994; Gorbsky, 1995). For chromosomes, it means alignment on the metaphase plate before entry into anaphase. To transduce the intrinsic heterozygosity of the genome in normal segregation at meiosis-I, the meiocytes of *R. spathacea* must co-ordinate their spindles with the metaphase-I ring. In principle, this co-ordination includes the checkpoints in relation to position and orientation of the chromosomes in the ring, kinetochore functions and the required forces in such a way that alternate chromosomes are segregated to the same pole. We discuss these aspects of *R. spathacea* in the context of contemporary information in bivalent forming, chiasmate organisms.

**Materials and Methods**

The material of *R. spathacea* was collected from the Botanical garden, Dept. of Botany, Bangalore University. Light microscopic studies were made by 2% aceticarmine squashes of suitable sized anthers. For TEM studies anthers were fixed in Karnovsky's reagent (3% glutaraldehyde + 1% paraformaldehyde), post fixed in osmium tetroxide, dehydrated in a ethanol series using 2% uranyl acetate as en bloc stain. Processed anthers were infiltrated and embedded in spurr's resin.

Figs. 1 - 6. Meiocytes of *R. spathacea*.
Figs. 1 and 2. A ring and a chain of 12 end to end paired chromosomes at metaphase-I, respectively.
Figs. 3, 4, 5 and 6. Metaphase-I, Anaphase-I, Telophase-I and a Dyad, respectively. Note the chromosomes poised for alternate segregation (fig. 3) and V-shaped configuration indicating a bipolar spindle (fig. 4).
Ultra thin sections (600 - 700°A) were obtained with an ultracut E. Reichert Jung Ultramicrotome and were post stained with uranyl acetate and lead citrate by conventional means prior to viewing on JOEL JEM 100 CX II Electron Microscope.

Results and Discussion

Synaptonemal Complex

Synaptonemal complex (SC) is both universal (Westergaard and Wettstein, 1972) and evolutionarily stable (Wettstein et al., 1984) in eukaryotes. Only two species, Schizosaccharomyces pombe (Kohli and Báehler, 1994) and Aspergillus nidulans (Egel-Mitani et al., 1982) are known to display meiosis without a detectable SC. Synaptonemal complex is a synapsis - exchange related organelle (Wahrman, 1981). In its fully expressed form, SC appears as a tripartite, ribbon-like structure and occurs throughout the length of the homologous chromosomes at pachytene (Westergaard and Wettstein, 1972; Gillies, 1973; Albini, 1994). SC disappears after pachytene except in cases where it acquires novel post-meiotic functions (Fil and Moens, 1973). After dissolution of SC, homologues remain connected by chiasmata (Jones, 1987). SCs are also implicated in chiasma interference (Maguire, 1990), chiasma maintenance (Maguire, 1977 & 1984), prophase-I chromosome condensation (Loidl, 1990) and resolution of interlocking (Weischman, 1986).

In R. spathacea, heterozygosity seems to have affected the formation of SC. A synegetic knot of SCs is seen in mid-pachytene (Stack and Soulhiere, 1984). On the basis of a positive correlation between genome size (1C DNA value) and SC lengths, and that 7.2 pg is the 1C DNA value for R. spathacea (Bennett and Smith, 1976), the expected total length of the SCs is 726 μm at pachytene (Stack and Soulhiere, 1984). All the SCs are attached to the nuclear envelope, coiled and are about 4 μm in length (Moens, 1972). According to Lin (1979a, b) diploid R. spathacea presents an example for modified SCs while Moens (1972) holds that coiling of SCs is an abnormality. The term 'coiled' is specifically used to differentiate from the counter twisting of SC lateral elements seen in other organisms. Whether the coiling of SCs in R. spathacea is a result of the pairing and recombination processes affected by the translocated segments or a modification to suit the needs of the situation remains to be ascertained.

Attempts of reconstructing pachytene nuclei have been unsuccessful in R. spathacea and SCs could not be followed throughout their length (Moens, 1972; McQuade and Wells, 1975; Lin, 1979a). As a result very little information is available regarding the nature of the translocated
segments as well as the involvement of SCs in the events of pairing, crossingover and chiasma formation. Presence of SCs in the terminal regions of chromosomes where crossingover and chiasma formation are also found indicates that the SCs may perform similar functions as in other bivalent organisms. The role attributed to SCs in the reductional segregation of sister centromeres (Moens and Spyropoulos, 1995), organisation of kinetochores in the first meiotic prophase (Counce and Meyer, 1973; Gillies, 1975) in bivalent forming organisms and physical orientation of kinetochores (Hawley and Arbel, 1993) may not be true in *R. spathacea* where the site of SC formation (telomeres) and kinetochores are spatially separated.

**Pairing and recombination**

Pairing and synapsis are two distinct processes, pairing is alignment of chromosomes in leptotene while synapsis is an association of homologues in the context of SC (Hawley and Arbel, 1993). Genomewide searches precede chromosome pairing (Smithies and Powers, 1986; Carpenter, 1987). Homology is recognised in two steps: presentation of the homologues and base pair matching between these (Loidl, 1990). Pairing depends on chromatin configuration (Maguire, 1984).

In comparison, the situation in *R. spathacea* is distinct. Though, squash preparations have not been useful for the analysis of earlier stages of pairing and synapsis (Walters and Gerstel, 1948; Desai, 1965; Lin and Paddock, 1973a; Stack and Soulliere, 1984; Garcia-Velazquez, 1991), their successful accomplishment is indicated by regularity of later events of first meiotic division (figs. 1 - 6). Only a small amount (about 1 %) of DNA is at the surface of the pairing chromosomes (Westergaard and Wettstein, 1972) implying an identical pattern of homologous chromosome folding to expose their pairing sites (Stern and Hotta, 1973). Considering that pairing and synapsis are restricted to very short terminal regions (20 - 30 % of total chromosomes length; Moses, 1968) in *R. spathacea*, a very highly efficient pairing system seems to be responsible for the observed success of meiosis in this organism.

In most eukaryotes, telomeres cluster resulting in a boquet stage at leptotene-zygotene (Schmit et al., 1995), and is considered to promote chromosome pairing (Zickler, 1977; Rasmussen and Holm, 1980; Dresser and Giroux, 1988; Loidl, 1990; Gilson et al., 1993; Schenthan et al., 1994). Telomeric association with one another, with nuclear matrix or nuclear envelope may promote chromosome pairing (Mason and Bressmen, 1995). Preferential recombination between chromosomes in subtelomeric sequences may help in pairing (Louis and Haber, 1990). In many
plant meiocytes, telomeres cluster but occupy a more wider area without giving the bouquet configuration (Moens, 1969; Fussein, 1987). In man (Schertzan, cf Heyting, 1996) and Saccharomyces pombe (Chikashige et al., 1994; Kohli, 1994) centromeres attach to the nuclear envelope slightly earlier than the telomeres. The end to end association of telomeres observed results in a linear array of chromosomes as in meiotic chromosomes of plants, yeasts (Klein et al., 1992; Kyrion et al., 1992) and in polytene chromosomes of Drosophila (Rubin, 1978). In pollen nuclei certain chromosomes appear to preferentially interact to form chain-like configurations (Ashley, 1979). The bouquet stage observed in many plant meioses is also seen in R. spathacea, (Stack and Soulliere, 1984), and is the most likely phase to initiate pairing interactions. Position of each chromosome in the ring is fixed (Lin and Paddock, 1973a). Therefore, association of any two telomeres in the ring has to be highly specific, since any nonhomology of pairing regions (chromosomes) aborts the pairing process itself (Radman and Wagner, 1993). Because SC formation can begin only when a critical distance of 2000 Å - 3000 Å is reached between the homologues (Moens, 1973; Gillees, 1975), it is also important that the pairing regions fulfill this requirement. Further, the pairing sites have to be protected from the effects of reciprocal recombination events which are also restricted to telomeric regions.

Crossingover is a fundamental part of sexual cycle (Nilsson et al., 1993). Crossovers in combination with intersister connections constitute chiasmata which are essential for disjunction of homologues at meiosis-I (Carpenter, 1994). In the absence of genetic recombination and chiasma formation, homologues dissociate at diplotene along with SC dissolution (Roeder, 1990). Pairing sets a limit for crossingover and crossingover to orientation and segregation (Sybenga, 1975). In R. spathacea, crossingover is possible in short paired regions, about 0.7 μm of the total chromosome length (Kollek, 1932). There may be none or up to 2 chiasmata per pair of chromosome arms at diplonema. Where chiasma has failed to occur in one or more pairs of arms, the ring becomes one or more chains (Lin and Paddock, 1973a, b).

Chiasmata correspond to points of reciprocal recombination between homologues (Murray and Hunt, 1993) and keep the homologues linked until the onset of anaphase-I (Wolf, 1994). Cohesion must be maintained along the chromosome arms to stabilise chiasma position (Darlington, 1934; Maguire, 1984 & 1995; Wolf, 1994). Chromosome cores contribute to sister chromatid cohesion required for chiasma maintenance and chromosome segregation (Moens and Spyropoulas, 1995). Chromosome segments not associated with strict homology would not support chiasma formation (Jenkins and Jimenez, 1995). Only some recombination events
yield crossovers while a majority are noncrossovers without any genetic exchange (FINK and PETES, 1984; CARPENTER, 1987; PERKINS et al., 1993). At least one crossover occurs on each homologous pair (ROEDER, 1990). Regular crossovers lead to normal segregation while irregular ones lead to aberrations (LEWIS and JOHN, 1966). Most organisms seem to have acquired a means to count their chiasmata, although how they do it is still uncertain (EGEL, 1995). Since ring formation depends on a minimum of 12 chiasmata, with the number of chains corresponding to the number of failures in chiasma formation (fig. 2), proper segregation in R. Spathacea is regulated by chiasma formation in the ring.

In the context of R. spathacea, the recombination and related events have to be viewed in an entirely different perspective. The end to end pairing points to the importance of telomeres in this process. Telomeres are an independent functional domain within the nucleus (GILSON et al., 1993) and are sites of recombination (LOUIS and HABER, 1990; RHODES and GILARDO, 1995). Since the telomeres are not known to carry any coding sequences, recombination could only be genetically inconsequential in these regions. Nevertheless, such 'sterile' crossovers also result in chiasmata which are required for the purpose of segregation. Even

![Fig. 7. Electronmicrograph of metaphase-I ring chromosome. Each chromatid shows a clearly differentiated kinetochore (K) region. Note the cohesion between the sister chromatids along the entire length of the chromosome. Bar represents 0.48 μm.](image)
recombination in nearby (subtelomeric) areas which tend to become homozygous over a period of time due to selection (Stebbings, 1971) would have the same effect. Thus, the distinction between the genetic effects of recombination and the mechanistic functions of chiasma is fully manifested in *R. spathacea*. Such recombination events with purely mechanistic functions seem to be necessary wherever heterozygosity in chromosomes needs to be protected. The crossovers in many mammals known to occur in pseudoautosomal segments (short homologous segments) in otherwise heterozygous X and Y chromosomes (Schempp et al., 1989) may be analogous to those occurring in the telomeres of *R. spathacea*.

The various functions of the telomere may be accomplished by the formation of an unusual structure, telosome (Wright et al., 1992). It would be worthwhile to know the molecular nature of telomeres in *R. spathacea* which seem to possess the versatility of facilitating the pairing of specific chromosome arms to form the ring as well as participating in recombination events without affecting the balanced lethal gene system.

**Nature of Spindle**

Spindle bipolarity is essential for chromosome segregation (Vernos and KarSENTI, 1996). Spindle structures and chromosome configurations in meiosis are broadly similar to mitosis but differ in certain important aspects (Reider et al., 1993). Astral spindles of animal cells are fundamentally different from anastral spindles of plants (Khodjakov et al., 1996). *Sciara coprophila*, a dipteran insect, shows a truly monopolar spindle (Wise, 1988) unlike the generally known bipolar ones. In *R. spathacea*, spindles of both I and II meiotic divisions are comparable to the other bivalent forming higher plants. The chromosome configurations at anaphase/telophase (figs. 4 and 5) are indicative of the bipolar nature of these spindles. The MT attachment to the two sister kinetochores facing opposite poles (figs. 8 and 9) further indicates the bipolar nature of the spindle at second meiosis.

**Kinetochores**

The terms kinetochore and centromere have been used synonymously (Sumner, 1990). Centromere is the site of kinetochore location (Sumner, 1990; Pluta et al., 1995). A ‘point centromere’ attaches a single MT as in *Saccharomyces cerevisiae*, while a ‘regional centromere’ in higher eukaryotes has multiple MT bases (Pluta et al., 1995). Centromeric activity
Fig. 8. Electronmicrograph of a PMC at metaphase-II. Bar represents 2.50 μm.

Fig. 9. Part of fig. 8 enlarged to show the ball and cup type of kinetochore on each of the sister chromatid (K) facing opposite poles indicating a bipolar spindle. About 6 - 8 kinetochore microtubules (KMTs) are seen (per section) attached to each kinetochore. Bar represents 0.37 μm.
could be either localised as in monocentric chromosomes or diffused as in holocentric chromosomes of certain organisms.

At the ultrastructural level, kinetochores of monocentric chromosomes of eukaryotes is either trilaminate or ball-and-cup type. Both these types have been recorded in different plant groups (GODWARD, 1985; BASKIN and CANDE, 1990). The higher plants so far examined have revealed a ball and cup type of kinetochore which appears as a dome shaped structure set at the surface of the chromosomes, and is attached to a relatively high number of MTs (BAJER, 1968; WILSON, 1968; MESQUITA, 1970; BRASELTON and BOWEN, 1971; BAJER and MOLE-BAJER, 1972; JENSEN and BAJER, 1973; ESPONDA, 1978; HANOAKA, 1981; RANGANATH and APARNA-RAU, 1997). According to MOENS (1979), a high number of KMTs offers flexibility to chromosomes during movement. Kinetochore of monocentric chromosomes of R. spathacea conforms to the ball-and-cup type mentioned above (figs. 7 & 9). Each sister chromatid of a metaphase-I ring chromosome shows a differentiated kinetochore region, measuring about 0.48 \( \mu m \) to 0.50 \( \mu m \) in width. The entire kinetochore (centromeric) region appears highly compact. The CEN value (centromere size; BLOOM, 1993) of the chromosomes as indicated by about 50 - 60 KMTs (10 - 12 per section observed) attached to kinetochore is comparable to the monocentric chromosomes of other higher plants examined so far. This similarity with the kinetochores of other higher plants in ultrastructural details indicates a functional similarity as well.

In meiosis-I, bivalents are properly oriented by chiasmata (RICKARDS, 1964). Homologues are required to show their sister centromeres facing opposite poles so that reduction division is achieved (OSTERGREN, 1951; SYBENGA, 1975; MAGUIRE, 1995). A regional centromere is an aggregate of microtubule bases with associated proteins (GODWARD, 1985; BLOOM, 1993). Recombinant DNA studies in yeast have shown that the MT sites have a specificity (FITZERALD-HAYES, et al., 1982). MT sites in the kinetochore have to appear in appropriate orientation on the metaphase chromosome for proper segregation (CAVALIER-SMITH, 1985). Improper orientation of the MT sites may be as disastrous to the chromosome as multiple centromeres are (HILL and BLOOM, 1989). Chromosomes with physical connections are multivalents (SYBENGA, 1973). Multivalents originate primarily in two ways, (a) translocation heterozygosity and (b) polysomy and in such systems metaphase configurations are important than the process of pairing itself (JOHN, 1990). In translocation heterozygotes, two special chromosomes form a balanced set i.e., only alternate chromosomes form a balanced set. In such cases coorientation is a matter of forces, not homology (SYBENGA, 1975). Selection for fertility leads to increased alternate orientation in multivalents (LAWRENCE, 1958) which
appears to be true in *R. Spathacea* also due to end to end pairing of chromosomes at meiosis-I. For the onset of anaphase the intertwining at chiasmata is removed by topoisomerase-II only after all the kinetochores with their attached MTs are in 'P' orientation (Murray and Szostak, 1985). Since only properly assembled kinetochores can interact with MTs, the eventual 'P' orientation of alternate chromosomes in the metaphase-I ring to one pole depends on events much earlier to the metaphase alignment of the ring.

The kinetochore disposition on the sister chromatids of a metaphase-I chromosome in *R. Spathacea* (fig. 7) is back to back i.e., facing opposite poles. In such situations where metaphase-I (meiotic) chromosomes look like mitotic metaphase chromosomes, only one of the sister kinetochores should be functional to achieve reductional division at meiosis-I while both should be active for chromatid separation during meiosis-II (fig. 9). The situation seems to be different from the available models explaining meiosis-I i.e., a) co-orientation of sister kinetochores in each homolog (Nicklas, 1977, 1997; Goldstein, 1981) or b) delayed centromere replication (Murray and Szostak, 1985). The consequences of such kinetochore disposition in meiotic chromosomes on chromosome segregation mechanics deserves to be thoroughly examined at both ultrastructural and molecular level. *Rhoeo Spathacea* and species of *Oenothera* with metaphase-I ring of chromosomes showing relatively more exposed kinetochores regions are good model organisms for such studies.

### Sister chromatid cohesion

Mechanistically, cohesion assures proper segregation in both mitosis and meiosis (Nicklas, 1974 & 1977; Reider, 1991). Dissolution of elements (cohesion proteins) holding sister chromatids together and the consequent segregation are a land mark of metaphase-anaphase transition (Miyazaki & Orr-Weaver, 1994). Cohesion is essential in meiosis, particularly in the pericentric regions throughout the first (reductional) division until anaphase-II (Maguire, 1990; Sekelesky and Hawley, 1995). Cohesion must be maintained along the chromosome arms to stabilize chiasma (Maguire, 1984).

The chromosome features of *R. Spathacea* display all the cytologically visible aspects of cohesion in both meiosis-I and -II. The release of sister chromatids from the cohesive forces is fully manifested at metaphase-II i.e., before entry into anaphase-II (figs. 8 & 9) unlike the sister chromatids tightly held by cohesion along the entire length of chromosome at meiosis-I (figs. 3, 4 & 7). Heterozygosity (translocations) seems to have no effect on the nature of cohesion between sister chromatids. Any invol-
movement of SC or SC-related proteins in cohesion is excluded since cohesion lasts until anaphase-II while SC disappears in prophase-I (MIYAZAKI and ORR-WEAVER, 1994). This aspect is more evident in *R. spathacea* where SC formation is restricted to terminal regions while cohesion is manifested along the entire length of chromosomes. Similarly, the role of organizing kinetochores of first meiotic chromosomes attributed to SC (COUNCE and MEYER, 1973; GILLIES, 1975) does not apply to the situation in *R. spathacea* where the SC and kinetochore are not in physical contact at all.

**Force transduction**

Movement of chromosomes during anaphase-A may be due to: (1) molecular motors at the kinetochore or (2) depolymerization of tubulin subunits from the plus ends of KMTs or (3) MT flux, 'reeling in' of MTs towards the spindle poles (WEIN et al., 1996 and the references therein). Since the chromosomes are in mechanical contact with the pole via MTs, it is reasonable to infer that the necessary force is generated at the pole or the kinetochore or even along the MTs connecting the chromosomes with the pole (SEMENOV, 1996). Kinetochore motility depends on its interaction with plus ends of MTs (CASSIMERIS et al., 1990). Thus, irrespective of the source of force production, it is essential that the MTs are in physical contact with the kinetochore to effect segregation. In *Sciara coprophila* (WISE, 1988) attachment of the MTs to the kinetochore immobilizes the paternal set of chromosomes rather than facilitating their movement. In (mitotic) metaphase, the chromosomes are uniformly located midway between the spindle poles i.e., where the sum of all the forces acting on each chromosome is zero (SEMENOV, 1996; VERNOS and KARSENTI, 1996). It is not known whether the polar ejection force (FULLER, 1995) observed in the astral spindles of animal cells is relevant in the context of anastral plant spindles where it is yet to be demonstrated (KHODJAKOV et al., 1996). Even in the former case, polar ejection force is terminated by metaphase so that it does not interfere with anaphase movement of chromosomes (KHODJAKOV et al., 1996).

In general, orientation of a centromere towards a pole remains stable only when a counter force (pull) is exerted by the centromere of the other homologue (NICKLAS, 1968 & 1971) and the chiasma attaching the partners (JOHN, 1990). If this counter force lapses, the chromosomes get an opportunity to reorient (SYBENGA, 1975). In achiasmate forms, NOD protein substitutes for chiasma (MCKIM and SCOTT-HAWLEY, 1995).

Due to end to end pairing of chromosomes in *R. spathacea*, metaphase alignment would be due to transduction of the required force in the
entire ring as a single unit unlike bivalent meiosis. Poleward forces acting on a bioriented metaphase chromosome stretch the kinetochore at an average force of 5 m dyn (NICKLAS, 1988). It is believed that the stretching of the underlying centromeric chromatin induces the kinetochore to activate the MT motors (YEN and SCHAAR, 1996). Centromere chromatin acts as a direct communication link between sister kinetochores (SKIBBENS et al., 1993). The similar sized chromosomes of *R. spathacea* are likely to have a comparable centromere size (CEN value) and hence the poleward force generated at these centromeres, six facing each pole (fig. 3), and the counter force generated by the chiasmata at telomeric regions may lead to a uniform tension on each chromosome facilitating the observed metaphase alignment of the ring at the mid cell zone.

**Chromosome segregation in a triploid**

Unlike the diploids (2n = 12), the triploid (3n = 18) revealed a metaphase-I ring of bivalents alternating with single chromosomes because of an additional alpha complex from an unreduced gamete (LIN and PADDOCK, 1978). This unusual configuration indicates that the chromosomes involved in bivalent formation pass through the stages of SC formation, pairing and recombination, as in bivalent meiosis. At the same time, one of the homologues in the bivalent has to simultaneously initiate interactions with two other chromosomes with which it shares partial homology to form the observed metaphase-I ring. Obviously, the biochemical pathways of both bivalent and ring formation processes are intact and expressed in equal propensities. Such triploid individuals offer an excellent opportunity to examine the cytogenetic events leading to bivalent formation and ring formation at the same time. That the spindle copes with the segregation of both bivalents and single chromosomes (alpha and beta sets of chromosomes) to different poles is a noteworthy aspect.

**Conclusions**

*Rhoeo spathacea* is an instructive example of an unusual meiotic system in which problems of chromosome segregation and maintenance of meiotic fidelity have acquired a new dimension. The occurrence of meiocytes in separate sex organs is considered as an ultimate expression of sexuality in organisms. Meiosis specific homologue pairing and recombination are the basis for genetic variation among the offspring. However, this consequence of meiosis is lost due to genetically neutral recombination events in the telomeres. By neutralising the genetic effects of
recombination and retaining only the mechanistic functions of chiasmata, translocation heterozygosity seems to have converted meiosis into an evolutionary burden on this organism. Thus, R. spathacea challenges the basic tenets of meiosis and represents a paradoxical situation in the sexuality of angiosperms.

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Meiosis-I in *Rhoeo spathacea* – A Contemporary View


Telomere Functions in the Translocation Heterozygote, *Rhoeo spathacea*

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Summary The permanent translocation heterozygote, *Rhoeo spathacea* shows a characteristic ring of all chromosomes at metaphase-I of male meiosis. Elimination of bivalent formation completely due to extreme heterozygosity poses a special problem for reductional division at meiosis-I where the maternal and the paternal sets need to be separated to different poles. Unlike bivalent meiosis, the functions of SC formation, pairing and recombination required for reductional segregation have to be fulfilled by only telomeres which are the only areas of contact among the chromosomes in the ring. The participation of telomeres in these events assumes significance in view of the balanced lethal gene system imposed by translocation heterozygosity, and the particular requirement that only alternate chromosomes segregated to the same pole results in fertile pollen. This paper throws some light on the telomere functions in *R. spathacea* in the light of our present understanding of telomere biology. The prospects of using *R. spathacea* as a model organism to dissect molecular aspects of several telomere related functions are also discussed.

Diploid *Rhoeo spathacea* (Swartz) Stearn of Commelinaceae, a complex heterozygote, shows a characteristic ring of all the twelve, end to end paired chromosomes at metaphase-I of male meiosis. The ring is interpreted as a consequence of translocations (Stack and Soulliere 1984) eliminating bivalent formation completely. Even in such an unusual situation, the organism maintains meiotic fidelity by effecting reductional segregation. At the heart of the entire process are the telomeres which are the only areas of contact among the chromosomes in the ring.

The term telomere, in greek for “end” (telos) and “part” (meros), was coined by Muller. Telomeres are the protein-DNA complexes found at the termini of eukaryotic chromosomes required for synthesis of ends of DNA molecules and several other important cellular functions (Biesmann and Mason 1992, 1997, Sandell and Zakian 1993, Gilson et al. 1993, Kirk et al. 1997, Lansdorp 1997). They form protective caps that stabilize chromosome ends and shield them from cellular surveillance system that monitors DNA damage (Zakian 1995, Wang and Blackburn 1997, Wynford-Thomas and Kipling 1997). Telomeres (unlike double strand breaks) do not activate DNA damage checkpoint proteins such as ATM and P53, nor do they serve as substrates for repair enzymes that degrade or ligate ends. Instead, they undergo telomerase mediated replication and then mask the chromosome end from DNA damage checkpoint (Smith and de Lange 1997). Telomeres prevent exonucleolytic degradation and arrest the gradual loss of DNA associated with replication of linear molecules by conventional DNA polymerases (Mirabella and Gartenberg 1997). Chromosomes lacking telomeres undergo end to end fusion, cause cell cycle arrest and extremely high loss rates (McClelton 1941, Zakian 1989, Sandell and Zakian 1993, McEachern and Blackburn 1996).

A minimum telomere length appears to be required to maintain structural integrity of chromosomes. Shortening beyond this point causes replicative senescence (Harley et al. 1997). Telomeres also play a role in premeiotic chromosomal movement (Chikashige et al. 1994) and transcriptional silencing (Telomere Position Effect) of genes placed nearby (Levis et al. 1985, Gottschling et al. 1992).
Telomeric DNA consists of species specific motifs of 5–8 base pairs in length that typically contain clusters of three or four G-residues. These sequence motifs are repeated in tandem forming long, double stranded regions of telomeric DNA (Presting et al. 1996, Konig and Rhodes 1997). In all vertebrates including humans, the most terminal DNA consists of extended (upto 100 kb) arrays of 5'(TTAGGG)3' repeats (Moyzis et al. 1988, Lejnine et al. 1995). In plants, the telomeric DNA sequence motif 5'(lMAGGG)3' was first characterised in Arabidopsis thaliana (Richards and Ausubel 1988) and several other plant species (Kenton et al. 1993, Fajkus et al. 1996, 1995). The highly conserved nature of telomeric DNA reflects aspects of telomere function that is conserved in widely divergent eukaryotes (Smith and de Lange 1997). In most organisms, telomeric DNA sequences are complexed with telomere binding proteins (Fang and Cech 1995, Henderson 1995). Telomere function depends on the proper formation of a specific DNA-protein complex and in yeast, alterations of this complex can reduce cell viability (Lundbald and Szostak 1989, Kyrion et al. 1992, Virta-Pearlman et al. 1996, Cooper et al. 1997). Though there have been no reports on the structure and functions of telomeres in R. spathacea, the similarity in functions as in other eukaryotes could be anticipated because of cytological observations of chromosome stability and boquet structure during first meiotic prophase. However, unlike bivalent forming organisms, in R. spathacea only the telomeres have to fulfil the functions of synaptonemal complex formation, pairing and recombination required for the segregation of alternate chromosomes (maternal and paternal sets) during reductional segregation.

Synaptonemal complex

Synaptonemal complex (SC) is a synapsis-exchange related organelle (Wahrman 1981) occurring throughout the length of paired, homologous chromosomes at pachytene (Westergaard and von Wettstein 1972, Kleckner and Weiner 1993, Albini 1994, Egcl 1995, Heyting 1996), However, in R. spathacea SCs are coiled and occur only at short paired (telomeric) regions of first meiotic chromosomes. According to Lin (1979), coiled SCs are an example of modification due to translocation heterozygosity. Moens (1972) considers coiled SCs as an abnormality. However, presence of SCs in short, terminal, paired regions of chromosomes in R. spathacea, where crossingover and chiasma formation also occur, indicates that they may function as those in other bivalent forming organisms.

Pairing

In most eukaryotes, clustering of telomeres at leptotene-zygotene results in a boquet structure
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(Fussell 1987, John 1990, Chung et al. 1990, Funabiki et al. 1993, Palladino et al. 1993, Schmit et al. 1995) which is considered to promote pairing (Zickler 1977, Rasmussen and Holm 1980, Dresser and Giroux 1988, Loidl 1990, Gilson et al. 1993, Scherthan et al. 1994, Mason and Biessmann 1995). In meiosis, homologs first align at telomeres instead of their homology by DNA sequences (Chikashige et al. 1997). The nonhomologous chromosomes caught between homologous chromosomes need to be moved out of the way to allow a pair of homologs to be aligned. It may be reasonable to bring together all chromosomes at the telomeres and then shuffle them to search for a homologous partner (Chikashige et al. 1997). The bouquet structure is also seen in *R. spathacea* (Stack and Soulliere 1984) and is the most likely phase for pairing interactions to take place. Because all the chromosomes of an organism have the same repeated arrays of a sequence at their ends (telomeric repeats), strong associations can occur between the telomeres of both homologous and nonhomologous chromosomes (Scott Hawley 1997). In *Tetrahymenas* (Kirk et al. 1997) and *Drosophila* (Cenci et al. 1997), telomere separation seems to require cis- and trans-acting functions. If the situation in *R. spathacea* is similar, then the requirement that each chromosome should take a fixed position in the ring to facilitate an alternate segregation during reductional segregation (Lin and Paddock 1973a) cannot be explained on the basis of pairing interactions between telomeric repeats alone. Considering that: a) any nonhomology between the pairing regions aborts the pairing process itself (Radman and Wagner 1993), b) chromosome segments not associated with strict homology would not support chiasma formation (Jenkins and Jimenez 1995), c) preferential recombination in the more variable subtelomeric sequences may help in pairing (Louis and Haber 1990) and d) only specific interactions between two particular telomeres could place chromosomes in the ring such that alternate segregation is favoured, involvement of DNA sequences other than telomeric repeats becomes inevitable in pairing and ring formation. This seems to be likely since telomeres have several structural elements near each other, each with its own cellular function (Biessmann and Mason 1992). The pairing process should also meet the requirement of a critical distance between pairing regions for SC formation (Moens 1973, Gillies 1975).

Recombination

Several types of recombination are known in eukaryotic genomes: a) illegitimate or nonhomologous recombination between sequences that share little or no homology (Tang 1994), b) nonreciprocal recombination or gene conversion (Petes and Pukkila 1993), c) ectopic recombination between dispersed homologous repeats positioned on nonhomologous chromosomes (Jinks-Robertson and Petes 1985, 1986, Lichten et al. 1987, Selva et al. 1995, Datta et al. 1996), d) transposition of nonhomologous segments under specific conditions of interspecific hybridity or other conditions of genetic or physiological imbalance (Syngen 1996) and e) interstitial telomeric repeats in plants, ciliates and vertebrates acting as hot spots of recombination (Katinka and Bourgain 1992).

Genetic recombination, also known as homologous or allelic recombination or merely exchange, refers to reciprocal crossovers between nonsister chromatids of homologous chromosomes in a bivalent. Chiasmata correspond to points of reciprocal recombination between homologs (Janssens 1909, Jones 1987, Murray and Hunt 1993, Carpenter 1994, Petes and Pukkila 1995) and keep the homologs linked until the onset of anaphase-I (Wolf 1994, Orr-Weaver 1996). In *R. spathacea*, crossingover is possible in short paired regions of about 0.7 μm of the total length of all the chromosomes (Koller 1932). There may be none or up to 2 chiasmata per pair of chromosome arms at diplotype. Where chiasma fails to occur in one or more pairs of arms, one or more chains result (Lin and Paddock 1973a, b). In the absence of genetic recombination and chiasma formation, homologs dissociate at diplotype along with SC dissolution (Roeder 1990). While chiasma is presumed to be a resolved recombination event (Orr-Weaver 1996), chiasma itself needs to be resolved before the onset of anaphase-I. Being the source of counter force obstructing the poleward kineto-
chores based forces, chiasma generates tension necessary for segregation of homologs (Ostergren 1951) and is tested by a tension related metaphase-I checkpoint (Orr-Weaver 1996, Nicklas 1997, Li and Nicklas 1997). Tension is essential for resolving chiasmata and allow the onset of anaphase-I. At least one crossover is necessary on each homologous pair (Roeder 1990) to meet the tension requirements.

Recombination and related events in *R. spathacea* have to be viewed in a different perspective. The involvement of telomeres is evident due to SC formation and pairing confined to chromosome ends. Pairing sets a limit to crossingover and crossingover to orientation and segregation (Sybenga 1975). Telomere, an independent functional domain within the nucleus (Gilson et al. 1993), is a site of recombination (Louis and Haber 1990, Rhodes and Gilardo 1995). Since the telomeres do not carry any coding sequences, recombination is genetically inconsequential in these regions. Nevertheless, such ‘neutral’ crossovers also result in chiasmata which are required for the purpose of segregation. Recombination even in subtelomeric areas, which tend to become homozygous over a period of time due to selection (Stebbins 1971), would have the same effect. Thus, the distinction between the genetic effects of recombination and the mechanism functions of chiasma is fully manifested in *R. spathacea*. Such recombination events with purely mechanism functions seem to be necessary wherever heterozygosity needs to be protected. The crossovers in many mammals, known to occur in pseudoautosomal segments (short homologous segments) in otherwise heterozygous X and Y chromosomes (Schempp et al. 1989), are analogous to those occurring in the telomeres of *R. spathacea*. A backup distributive segregation system disjoins nonrecombinant homologs in some organisms (Nilsson-Tillgren et al. 1986, Scott Hawley et al. 1993). Such a system would not ensure the required alternate segregation from chains of chromosomes formed due to failure of recombination and chiasma formation in one or more pairs of chromosome arms during meiosis-I in *R. spathacea*.

A model organism

*Rhoeo spathacea* is a widely known but least understood complex heterozygote which can be used to study several molecular processes without the restrictions imposed by bivalent formation. For instance, in the absence of bivalent formation meiotic (metaphase-I) chromosomes in the ring look like mitotic metaphase chromosomes with their kinetochore/centromere regions being open and freely accessible for molecular examination. Rare instances of bivalent formation (Wimber 1968) indicate that translocation heterozygosity is a derived condition. Consequently, the cell cycle checkpoints monitoring meiosis-I need to be suitably modified to achieve a successful reductional segregation from the metaphase-I ring. Thus, several mechanism and molecular events known to be crucial for the success of bivalent meiosis could also be tested for their occurrence or adoption with suitable changes.

**Cell cycle checkpoints**

The neutral crossovers occurring in the telomeric regions and the unusual metaphase-I ring of chromosomes from which alternate chromosomes have to be segregated to the same pole offer scope to dissect the metaphase-I checkpoints i.e., the tension related (Ostergren 1951, Orr-Weaver 1996, Nicklas 1997) and the chromosome distribution (Li and Nicklas 1997) checkpoints, respectively.

**Pairing and recombination**

The ability of exchanges enhancing or decreasing the chances of successful chromosome segregation has been noted by several workers. It would be worthwhile to know the molecular nature of telomeres in *R. spathacea* which facilitate the pairing of specific chromosome arms to form a
metaphase-I ring as well as recombination events necessary for segregation without affecting the balanced lethal gene system enforced by translocation heterozygosity. Distal exchanges are less effective in ensuring proper segregation in human meiosis-I (Lamb et al. 1996, Koehler et al. 1996), due to premature loss of chiasmata in these regions (Orr-Weaver 1996). The possibility of exchanges themselves being inadequate in enhancing segregation, perhaps due to the decreased binding capacity of the chiasma binder at the telomeres, has also been pointed out (Boscom-Blackburn et al. 1997 and the references therein). In contrast, the success of reductional segregation in R. spathacea depending entirely on exchanges in telomeres may unravel the significance of position of exchanges in meiosis-I.

Telomere structure and function

Identification of telomeric DNA sequences in R. spathacea is urgently required and could be the basis for examining the involvement of telomeric protein like Ndj 1 known to participate in both homolog synopsis and meiotic chromosome segregation in S. cerevisiae (Conrad et al. 1997) and the unusual structure telosome (Wright et al. 1992) in pairing, recombination and segregation.

Telomerase

Telomerase is synthesized by nearly all organisms with nucleated cells (Giedri and Blackburn 1996). The precise makeup of the enzyme can differ from species to species, but each version possesses a species specific RNA template for building telomere repeats. Without telomerase, the chromosomes would shorten with every cell division eventually disrupting the genes (Barinaga 1997). Inhibition of telomerase activity by antisense template RNA leads to shortening of the telomeres to a critical length and to cell death (Feng et al. 1995). In human cells, elongation of telomeres by introduction of oligonucleotides (Wright et al. 1996a, b) or inhibition of telomerase by chromosome transfer (Ohmura et al. 1995) or chemicals (Stahl and Blackburn 1996) results in a prolonged life span or loss of cell viability, respectively. However, telomere elongation by telomerase is not universal. In Drosophila melanogaster, retrotransposable element HeT-A and TART are attached to chromosome termini and can be acquired by broken ends. In some dipteran insects and some Allium species where telomerase is permanently lost, the consequential loss of telomeric sequences and the telomere binding proteins expose the naturally occurring double strand DNA. Stimulation of recombination by these ends of DNA (like DSBs) may act as an alternative or backup mechanism for telomere elongation (Biessmann and Mason 1997 and the references therein). Roth et al. (1997) have also reported similar results in the mosquito, Anopheles gambiae. Report of alternative mechanisms for telomere maintenance (ALT) in human tumors in vivo (Bryan et al. 1997a) has made it necessary to consider circumventing ALT pathways also, along with antitelomerase drugs envisaged in cancer therapy (Lundbald 1997). The presence of ALT can be deduced from the combination of no detectable telomerase activity in the TRAP assay and telomeres that have a characteristic heterogeneity of length ranging from short to much longer than normal as determined by terminal restriction fragment (TRF) agarose gels (Bryan et al. 1997b). Notwithstanding that germline cells of euarkyotes are expected to show telomerase activity, demonstration of telomerase activity in meiocytes of R. spathacea would conclusively establish the nature of recombination in the telomeric regions.

Genome evolution

In the long arm of human chromosome 2, two inverted blocks of telomeric repeats mark the site of ancient telomere to telomere fusion that led to reduction of chromosome number from 24 to 23 between great apes and man (Ijdo et al. 1991). Whether or not the karyotype development in R. spathacea, with multiple and complex translocations requiring breakage and fusion, involves 'telomere fusion motifs' indicating translocation sites, forms an interesting aspect of study.
Evolutionary considerations

Crossingover is a fundamental part of the sexual cycle (Nilsson et al. 1993) and contributes to genetic diversity by ensuring variation among the products of meiosis (Murray and Hunt 1993). Though neutral recombination events in telomeres ensure the success of reductional segregation, the purpose of meiosis as a source of genetic variation is lost in *R. spathacea*. By neutralising the genetic effects of recombination and retaining only the mechanistic functions of chiasmata, translocation heterozygosity seems to have converted meiosis into an evolutionary burden on this organism. Thus, *R. spathacea* represents a paradoxical situation in the sexuality of organisms where the classical view of meiosis, as a source of genetic variation, does not apply.

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