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Synaptonemal complex formation in spermatocytes of the autotriploid rainbow trout, *Oncorhynchus mykiss* (Pisces, Salmonidae)

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Meiotic cells of triploid male rainbow trouts were analyzed by a surface-spreading SC technique in order to show the process of chromosome synapsis. At zygotene the formation of SCs involved, almost exclusively, two sets of lateral elements (LEs), and the remaining set of LEs presented different synaptic configurations involving one, two, three, or four LEs. The absence of SCs involving more than two LEs from mid- to late pachytene indicates that the multivalents produced by synapsis involving more than two LEs, are eliminated before the end of pachytene; thus, the mechanism of chromosome pairing in triploid male rainbow trouts produces, almost exclusively, bivalents with a probable extensive nonhomologous synapsis involving the extra set of chromosomes.

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Salmonidae is currently the most important group of fish used in cold-water aquaculture projects, and among the species of this family the rainbow trout is the most widespread one, with populations inhabiting waters on all continents except Antarctica (HERSHBERGER 1992). Triploid rainbow trouts have been extensively produced and used in aquaculture programs (CHOURROUT 1986). The analysis of triploid fish demonstrated that triploid females never mature, whereas triploid males develop typical secondary sexual characteristics and undergo the same hormonal changes as maturing diploids (LINCOLN and SCOTT 1984). Triploids occasionally produce spermatozoa, but the offspring produced from haploid eggs fertilized with such sperm do not survive past hatching, probably due to the occurrence of aneuploidy in male germinal cells (LINCOLN and SCOTT 1984; BENFEY et al. 1986).

Studies of the synaptonemal complex (SC) in fish are limited (PETERSON et al. 1994; OLIVEIRA et al. 1995, and references therein), and many aspects, such as SC behavior in polyploids, have not been investigated. Thus, the aim of the present investigation was to study the meiotic cells of triploid male rainbow trouts by a surface-spreading SC technique in order to show the process of chromosome synapsis in these fishes.

Material and methods

Five triploid males of the rainbow trout *Oncorhynchus mykiss* were used in the present study. They were obtained from a domesticated stock maintained at Estação Experimental de Salmonicultura of the Instituto de Pesca de São Paulo, Campos do Jordão, State of São Paulo, Brazil. Triploidy was induced by heat shock as described by CHOURROUT (1986). Fishes were screened by taking a blood sample to test the diameter of erythrocyte nuclei. Mitotic chromosome preparations from kidney tissue were obtained according to FORESTI et al. (1993).

A surface spreading method modified for the study of fish cells (OLIVEIRA et al. 1995) was used for SC analysis. Briefly, dissected testes were placed in Hanks' solution for cleaning and minced in a few drops of Hanks' solution over an inverted Petri dish covered with a parafilm layer. One drop of a cell suspension, two drops of fixative (4 % w/v paraformaldehyde, 3.4 % w/v sucrose), and four

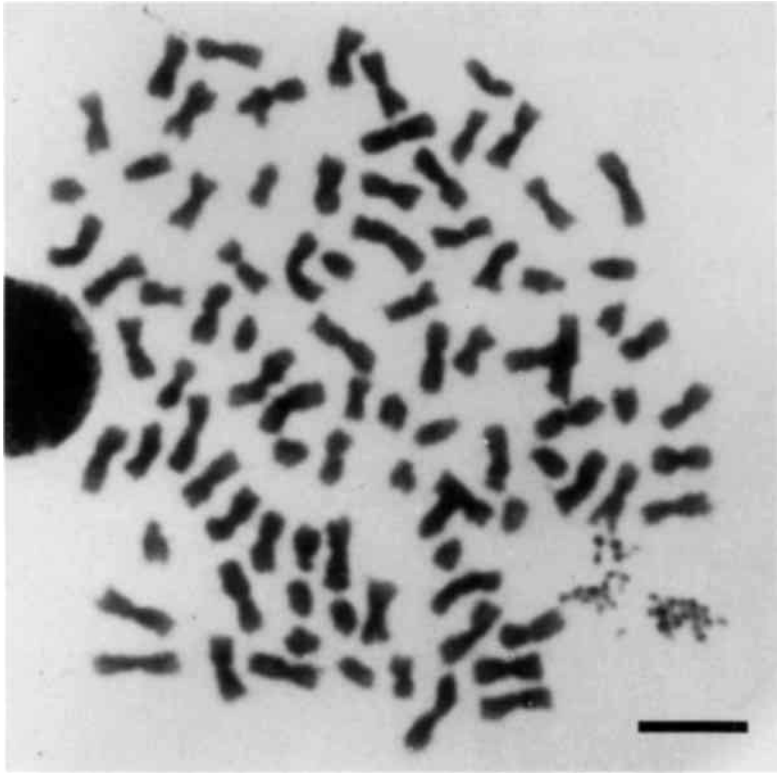

Fig. 1. Somatic metaphase of a rainbow trout specimen with 3n = 90 chromosomes stained with Giemsa. Bar = 5 μm.

drops of 1 % Lipsol (alkaline laboratory cleaning detergent) were mixed on a slide coated with plastic (Falcon). After 1 to 7 min (or by monitoring the cells under a microscope), 4 more drops of fixative were added to the mixture. The slides were then laid flat under a protective paper box and allowed to dry overnight, gently rinsed four times in a 0.4 % (v/v) Photo-Flo 200 (Kodak) solution, 30 s each time, and left to dry in a vertical position. The slides were silver-stained (HOWELL and BLACK 1980) for 6–12 min at 60°C. Excessive precipitation of silver nitrate was removed from the slides by cleaning them for 5–30 s in a solution of 0.1 % (w/v) periodic acid (DENTON 1989). For the electron microscope studies the plastic film was floated in tap water and picked up from below with 100 mesh copper grids. The grids were examined at 80 kV with a Phillips EM301 electron microscope.

Results

Mitotic chromosomes

Mitotic chromosome analysis of triploid rainbow trout (Fig. 1) showed that the chromosome number ranged from 3n = 89 to 3n = 94 (the modal number was 3n = 90) among cells of the individual and among cells of different individuals; however, the number of chromosome arms was constant (NF = 156). Cells with a low chromosome number presented a high number of biarmed chromosomes and cells with high chromosome number presented a high number of uniarmed chromosomes; thus, the occurrence of different chromosome numbers is due to Robertsonian rearrangements present among the cells of this species.

Synaptonemal complexes

Electron micrographs of 83 spermatocyte nuclei from leptotene to pachytene were obtained. LE

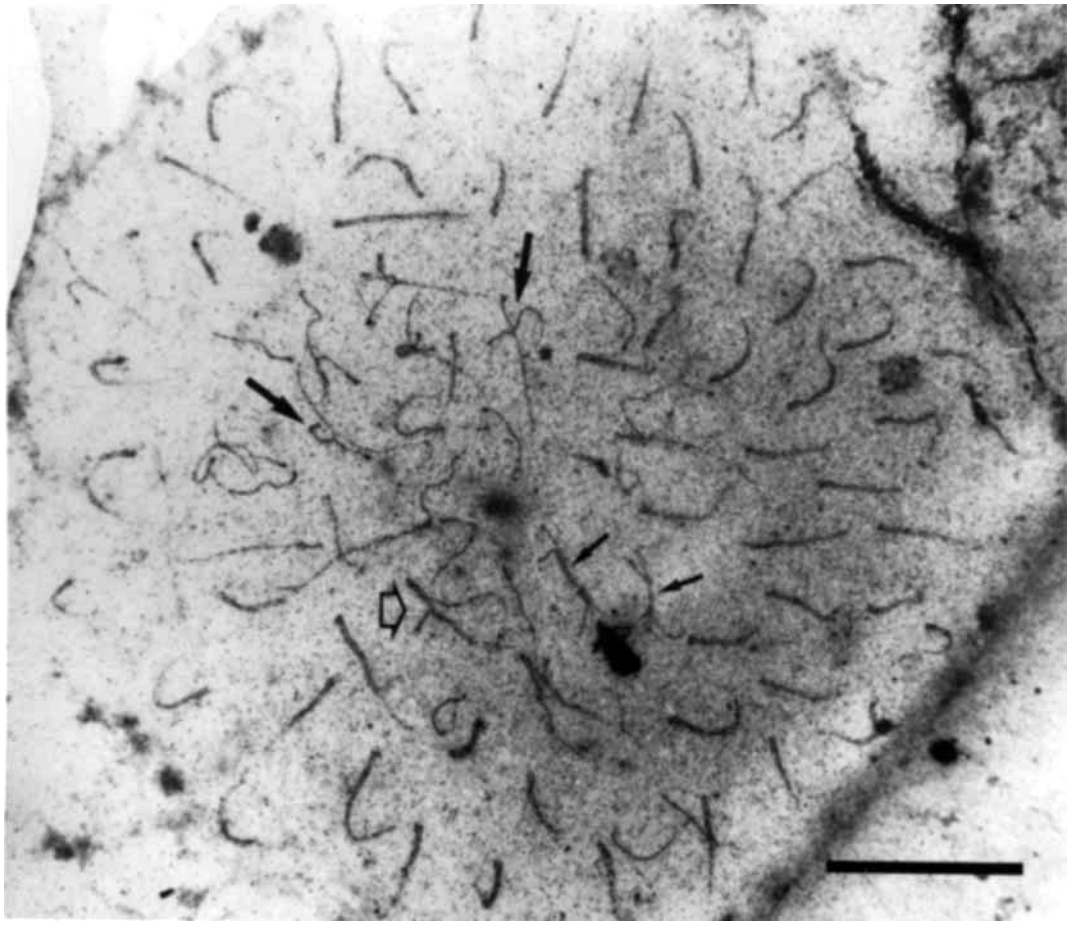

Fig. 2. Electron micrograph of a late zygotene nucleus with several univalents, presumed nonhomologous LEs at the beginning of the process of synapsis (large arrows) or in a more advanced stage of synapsis (small arrows), and three presumed nonhomologous LEs shaping a trivalent (open arrow). Note the ends of many LEs clustered in a specific region of the nucleus. Bar = 3 μm.

formation in triploid male rainbow trouts begins during leptotene near the telomeric region of each chromosome arm, which is signaled by the presence of attachment plaques. The number of LE pieces in this stage was about 156, which is in accordance with the presence of 156 chromosome arms. From early to middle zygotene the ends of the LEs were arranged in a closely restricted region of the nuclei giving origin to a partial bouquet configuration. In middle and late zygotene nuclei (Fig. 2) and in pachytene nuclei, it was observed that the extra set of LEs formed by univalents, bivalents, and multivalents remained in a closely restricted region of the nuclei.

The formation of LEs and the synapsis of the centromeric region of the biarmed chromosomes only occur during pachytene. Thus, from leptotene to zygotene each SC observed was defined as an "SC piece" since it could represent the synapsis of the two homologous chromosome arms belonging to one biarmed chromosome pair. Or it could represent the synapsis of two homologous chromosome arms belonging to one uniarmed chromosome.

The formation of SC stretches during zygotene involved, almost exclusively, two sets of LEs (Fig. 2). In the remaining set of LEs, we identified: (a) unsynapsed LEs as may be observed in Fig. 2;

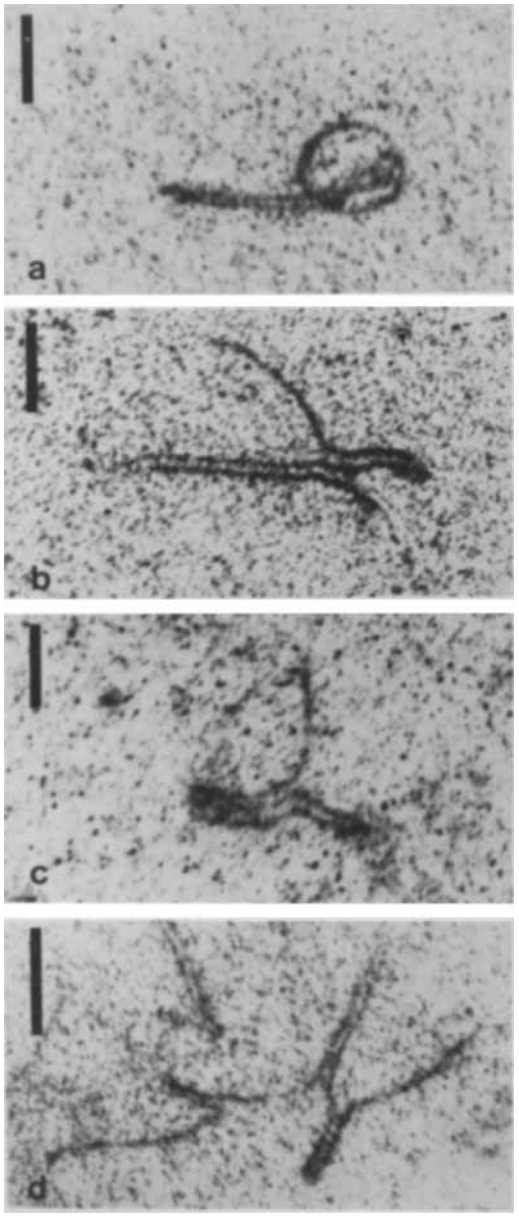

Fig. 3a–d. Electron micrographs of triploid male rainbow trouts SCs showing details of the synapsis involving the third set of chromosomes. **a** Univalent LE showing a U-shaped configuration but with the centromeric region unsynapsed. **b** Three presumed nonhomologous LEs synapsing as couples in different segments and with a region with a double synaptonemal complex. **c** A trivalent shaped by three presumed nonhomologous LEs synapsed as an almost full double SC. **d** Four presumed nonhomologous LEs synapsing as couples in three different segments. Bars = 1 μm.

(b) LEs showing a partial (Fig. 3a) or a full U-shaped configuration; (c) SCs formed by two presumed nonhomologous chromosomes at the beginning (Fig. 2) and during the course of the synaptic process; (d) three LEs synapsing as couples in different segments (Fig. 2 and 3b); (e) three LEs synapsing as couples in different segments, presenting a region with a double SC (Fig. 3b); (f) trivalents composed of three LEs synapsed as an almost full double SC configuration (Fig. 3c); and (g) four LEs synapsing as couples in three different regions (Fig. 3d).

In a quantitative analysis of 15 late zygotene nuclei from triploids, the mean values ($\bar{x}$) obtained as a result of counting each arrangement of LEs and/or SC pieces were: almost fully synapsed SC pieces, $\bar{x} = 54$ (ranging from 52 to 60) (the centromeric region was not considered since it was visible only in pachytene); unsynapsed LEs, $\bar{x} = 36.3$ (ranging from 25 to 46); LEs showing a partial or a full U-shaped configuration, $\bar{x} = 1.0$ (ranging from 0 to 4); SCs formed by two presumed nonhomologous chromosomes, $\bar{x} = 4.9$ (ranging from 1 to 10); three LEs synapsing as couples in four different segments, $\bar{x} = 0.2$ (ranging from 0 to 1); four LEs synapsing as couples in different segments, $\bar{x} = 0.1$ (ranging from 0 to 1). Other configurations than described above were not found in the 15 nuclei analyzed. Although all these unusual configurations were found more frequently in late zygotene, some similar figures were also observed in mid zygotene and early pachytene.

During early pachytene the central regions of the biarmed chromosomes were progressively synapsed, giving origin to SCs with two attachment plaques in which, in some cases, the centromeric regions were particularly evident. The number of SCs with two attachment plaques increased from early to late pachytene, and the frequency of unsynapsed LEs and of SCs involving three or more LEs was progressively reduced.

Discussion

The processes of LE formation during leptotene, the process of synapsis of the chromosome arms during zygotene, and the synapsis of the centromeric regions of biarmed chromosomes during pachytene in triploid male rainbow trouts are very similar to those observed in diploid males (OLIVEIRA et al. 1995).

Nuclei of diploid male rainbow trouts at late zygotene presented 51 SC pieces and two unsynapsed LEs, which probably correspond to the X and Y chromosomes (OLIVEIRA et al. 1995). Triploid male rainbow trouts presented at least 52 SC pieces, suggesting that synapses between the X chromosomes occur more frequently than X–Y synapses. The analysis of the behavior of sex chromosomes has been difficult due to the presence of many unsynapsed LEs, but the above hypothesis is in accordance with the observations of SOLARI et al. (1991) that in triploid female chickens the synapsis between the two Z chromosomes is much more frequent than synapsis between the Z and W chromosomes. Moreover, the hypothesis of the existence of a preferential synapsis between the two X chromosomes in triploid male rainbow trouts is in accordance with the data from gene segregation in tetraploid rainbow trouts showing a preferential pairing of homologous chromosomes (DITER et al. 1988).

In late zygotene nuclei of diploid male rainbow trouts, which present 104 chromosome arms ($2n = 56$ to $2n = 68$), the maximum number of SC pieces observed was 51 (OLIVEIRA et al. 1995). The presence of 52–60 complete SC pieces in late zygotene nuclei and the low number of univalents found in pachytene of triploids suggest that the elements of the additional set of chromosomes synapsed amongst themselves. The analysis of nuclei from late zygotene to early pachytene showed a particular pattern of chromosome synapsis involving different combinations among the elements of the third set of LEs, suggesting an extensive synaptic process among different nonhomologous chromosomes in triploid male rainbow trouts.

RASMUSSEN (1977) and LOIDL et al. (1991) pointed out that homology is not an indispensable precondition for SC formation, but rather that chromosomes probably are engaged in nonhomologous synapsis if no homologous partner is available. Thus, according to the cited authors, the synapsis has a tendency to be maximized irrespective of homology, supporting the concept that SC is a structure that may connect chromosomes nonspecifically. The finding of the presumed nonhomologous synapsis involving the third set of chromosomes of triploid male rainbow trouts is in accordance with this hypothesis.

There is no uniform pattern of chromosome pairing in triploids of different species, and the main variations occur in the extent and in the persistence of pairing among the three axes of each homologous set (SOLARI et al. 1991). The present data show that the mechanism of synapsis in triploid male rainbow trouts is similar to the one described for *Bombyx mori* (RASMUSSEN 1977) since only bivalents are found in late pachytene nuclei of triploids of both species. The main differences observed for LE behavior in the chromosomes of triploid male rainbow trout as compared with other examples in the literature are the apparent absence of alignment of the three homologous LEs as observed in the triploids (RASMUSSEN 1977; LOIDL and JONES 1986; VINCENT and JONES 1993; THOMAS and THOMAS 1994) and the absence of complete trivalent formation as described in *Coprinus cinereus* (RASMUSSEN et al. 1981) and *Gallus domesticus* (SOLARI et al. 1991).

Rainbow trout represents a species originating from an autotetraploid event in an ancestor of the group (ALLENDORF and THORGAARD 1984), and there are synapses involving three or four LEs in diploid rainbow trouts (OLIVEIRA et al. 1995). Thus, it is possible to hypothesize that the unusual synapsis behavior among LEs in triploid male rainbow trouts is due to the presence of homologous chromosome segments in the third set of chromosomes. Such homologous segements could be responsible for the occurrence of initial synapsis among the third haploid set of chromosomes. These initial synapses do not permit the formation of trivalents and also transport the other nonhomologous segments to a nonhomologous synapsis. Alternatively, the occurrence of nonhomologous pairing among the elements of the third set of LEs may be due to spatial distribution, since almost all of these elements remain in a closely restricted area of the nuclei from middle zygotene to pachytene.

The presence of trivalent segments found in zygotene and in early pachytene nuclei and their absence in late pachytene suggest their elimination before the end of the pachytene stage, such as observed in *B. mori* (RASMUSSEN 1977). The presence of mechanisms of trivalent elimination is not widespread among the organisms; species with very long chromosomes such as *Allium sphaerocephalon* (LOIDL and JONES 1986), *Crepis capillaris* (VINCENT and JONES 1993), and *Lolium multiflorum* (THOMAS and THOMAS 1994) and some species with small chromosomes such as *Coprinus cinereus* (RASMUSSEN et al. 1981) do not present mechanisms for trivalent elimination during prophase I.

The analysis of triploid spermatozoa of rainbow trout by flow cytometry demonstrated that these cells present DNA content values intermedi-

ate between haploid and diploid values (BENFEY et al. 1986). This could be explained by normally segregating bivalents and a random segregation of a third set of univalents or, alternatively, by the simple random segregation of three chromosome sets. The presence in late pachytene nuclei of almost exclusively bivalent SCs suggests that the first hypothesis is the correct one. However, in addition to the random segregation of some elements of the third set of univalents there is probably the formation and segregation of chromosome fragments which may result from crossing-over involving nonhomologous chromosomes.

Further studies of artificial tetraploids and hybrids would be very interesting to test the hypothesis of nonhomologous synapsis and the existence of homologous chromosome segments among the members of the haploid chromosome set of rainbow trout. Additionally, studies on different triploid fish species will possibly provide useful information about chromosome segregation in this animal group.

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