NORs inheritance analysis in crossings including individuals from two stocks of rainbow trout (*Oncorhynchus mykiss*)

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Silver nitrate staining of rainbow trouts (*Oncorhynchus mykiss*) chromosomes, for the identification of the nucleolar organizing regions (NORs), revealed that in individuals from Núcleo Experimental de Salmonicultura de Campos do Jordão (Brazil) NORs were located in the long arms of a submetacentric pair while in specimens from Mount Shasta (USA) NORs were located in the short arms of a submetacentric pair. Cytogenetic analysis of the offspring, obtained through artificial crosses including individuals from both stocks, allowed the identification of NORs in two submetacentric chromosomes, one in the short arms and the other in the long arms, confirming the effectiveness of the hybridization process. Complementary results obtained using the FISH technique with 18S and 5S rDNA probes showed that NOR-bearing chromosomes exhibited a cluster of 5S genes located in tandem with the 18S gene cluster in both stocks. The results allow us to suggest that the difference in NOR-bearing chromosomes found between the two stocks is likely to be due to a pericentric inversion involving the chromosome segment where 18S and 5S rDNA genes are located. The presence of ribosomal genes in the long arms of a submetacentric chromosome is apparently a particular characteristic of the rainbow trout stock of Campos do Jordão and might be used as a chromosome marker in studies of controlled crosses in this species.

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Cytogenetic studies in fish have shown that they frequently present nucleolar organizing regions (NORs) with large size polymorphisms (FORESTI et al. 1981), but the number and position of NORs are generally specific of a given population or species (VÊNERE and GALETTI Jr 1989; MOREIRA-FILHO and BERTOLLO 1991; MAISTRO et al. 1998).

Silver staining of the NORs and fluorescent in situ hybridization (FISH) with 18S rDNA gene probes have provided similar results in fish chromosomes (REED and PHILLIPS 1995; CASTRO et al. 1997; ROSSI et al. 1997). FUJIWARA et al. (1998) showed that, in rainbow trout (*Oncorhynchus mykiss*), a single chromosome pair contains the major rDNA genes (18S + 5.8S + 28S) and two chromosome pairs contains the minor rDNA genes (5S). In one chromosome pair, both major and minor rDNA genes, are contiguously arranged.

Cytogenetic analysis of rainbow trout specimens from Mount Shasta (California, USA), using the Ag-NOR banding technique demonstrated that NORs are located at an interstitial position in the short arm of a submetacentric chromosome pair (PORTO-FORESTI et al. in press), as described in other populations of this species (SCHMID et al. 1982; PHILLIPS and IHSSEN 1985; MAYR et al. 1986; UEDA and KOBAYASHI 1988; LLOYD and THORGAARD 1988). In rainbow trout specimens sampled at Núcleo Experimental de Salmonicultura de Campos do Jordão (São Paulo, Brazil), OLIVEIRA et al. (1996) observed that NORs were interstitially located in the long arm of a submetacentric chromosome pair. According to OLIVEIRA et al. (1996) the differences in NOR location found in rainbow trout stocks are likely to be due to the occurrence of chromosome inversions or translocations.

In the present study, hybridization experiments were conducted with fish from the stocks of Núcleo Experimental de Salmonicultura de Campos do Jordão and Mount Shasta and the major and minor ribosomal genes were analyzed in their offspring by the silver staining technique and FISH with 18S and 5S probes.

MATERIAL AND METHODS

Two rainbow trout stocks were used: one from Núcleo Experimental de Salmonicultura de Campos do Jordão (São Paulo, Brazil) which has been kept isolated for more than 20 years, and a second stock

recently imported by Salmonicultura N.R. (Sapucaí Mirim, Minas Gerais, Brazil) from the region of Mount Shasta (California, USA). Artificial crossings were conducted with individuals from these two stocks and the embryos and fries were cultivated in isolated tanks at Núcleo Experimental de Salmonicultura de Campos do Jordão until cytogenetic analyses.

Parental and F_1 specimens were submitted to cytogenetic analysis by direct cell suspension with kidney cells (FORESTI et al. 1993). Before sacrifice, the animals were inoculated with a yeast cell suspension to increase the number of metaphase cells (LOZANO et al. 1988). The procedure used to identify the nucleolar organizer regions (NORs) was that originally described by HOWELL and BLACK (1980). For FISH analysis, two rDNA sequences, 18S (about 1800 base pairs) and 5S (about 550 base pairs), isolated from *Oreochromis niloticus* and prepared by Dr. Claudio Oliveira, were used. Probes were labeled with biotin-14-dATP using a commercially available nick translation kit (Bionick™ Labeling System, Gibco). For in situ hybridization, the slides were incubated in RNAse (100 ng, 2 × SSC) at 37°C for 1 h and dehydrated in an ethanol series (75 %, 80 %, 95 %) at − 20°C. Chromosomal DNA was denatured by immersing the slides for 5 min at 70°C in 70% formamide, 2 × SSC. The slides were than dehydrated in an ethanol series (75 %, 80 %, 95 %) at − 20°C. The hybridization mixture was composed of 50% formamide in 2 × SSC, 10% dextran sulphate, 500 µg/ml biotinylated DNA probe. After 10 min denaturation at 75°C, 15 µl of hybridization mixture was applied to each slide under a sealed cover glass. Hybridization was performed overnight in a moist chamber at 37°C. The slides were washed, under different conditions, for 20 min in formamide 50%, 2 × SSC at 37°C or 42°C, twice for 10 min in 2 × SSC (37°C or 42°C) and twice for 10 min in 4 × SSC (37°C or 42°C). Hybridization was detected with fluorescein-labeled avidin (FITC) (Oncor) incubated at 37°C in a moist chamber for 30 min. Subsequently the slides were washed twice in 1 × PBD and once in 1 × PBS for 3 min each. The chromosomes were counterstained with propidium iodide/antifade solution (Oncor). In some slides the 5S rDNA signal was enhanced by incubation with anti-avidin antibody (Oncor) followed by the application of fluoresceinated avidin. Metaphases were examined under fluorescent microscopy. For chromosome prints a Kodak Gold Plus ISO 400 film was used.

RESULTS AND DISCUSSION

The Ag-NOR staining of the chromosomes of the specimens cultivated in Núcleo Experimental de Salmonicultura de Campos do Jordão showed that NORs were located in the long arms of a submetacentric chromosome pair (Fig. 1a) as described by

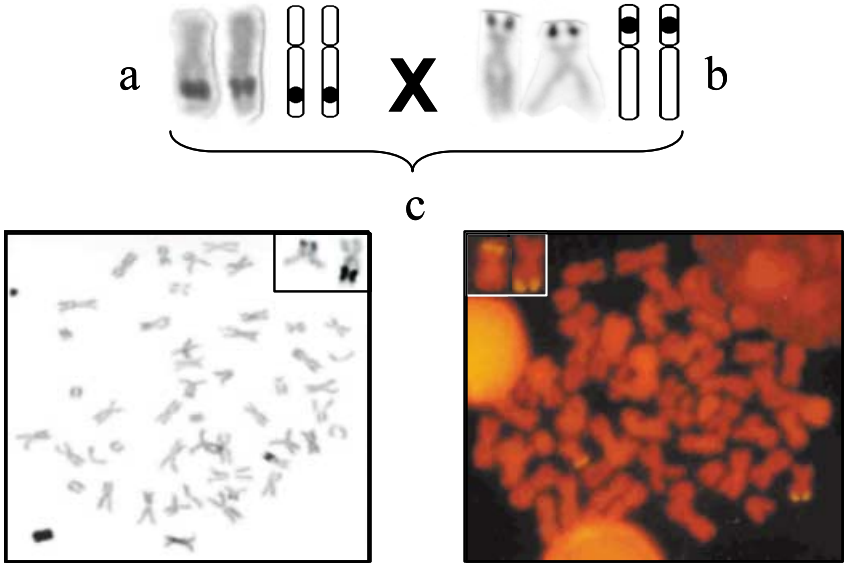

Fig. 1a–c. Rainbow trout (*Oncorhynchus mykiss*) NOR-bearing chromosomes. **a** Silver-stained chromosomes of a specimen from Núcleo Experimental de Salmonicultura de Campos do Jordão, São Paulo, Brazil. **b** Silver-stained chromosomes of a specimen from the Mount Shasta region, California, USA. **c** Somatic metaphases of a F_1 hybrid stained with silver-nitrate and with the FISH technique with the 18S rDNA probe respectively. In the insets, the NOR-bearing chromosomes.

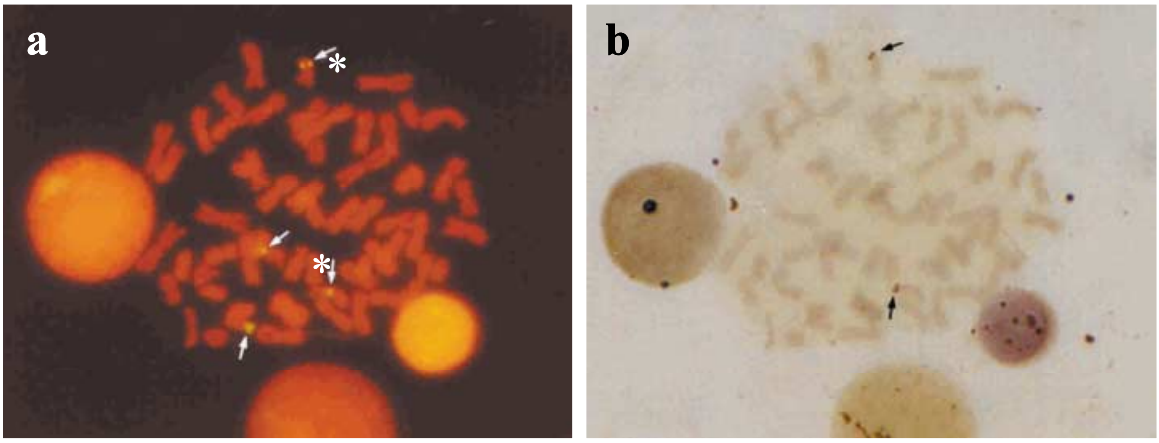

Fig. 2a–b. Sequential staining of a somatic metaphase of F_1 rainbow trout (*Oncorhynchus mykiss*) specimens. **a** Silver-stained metaphase, the arrows point to the two chromosomes with NORs, in the inset, a detail of the NOR-bearing chromosomes. **b** Metaphase stained by the FISH technique with a 5S probe; the arrows indicate the presence of four chromosomes with 5S genes, in the inset, a detail of the 5S-bearing chromosomes. The asterisks show the chromosomes with both NORs and 5S genes.

OLIVEIRA et al. (1996). In the Mount Shasta sample, NORs were observed in the short arms of a submetacentric chromosome pair (Fig. 1b). The FISH technique using 18S rDNA probes applied in the chromosomes of specimens from both stocks evidenced signals at a single chromosome pair, at the same position of the NORs previously detected using the Ag-NOR banding technique (Fig. 1).

Among the individuals from the F_1 generation, NORs were identified in two submetacentric chromosomes. In one of these chromosomes, NORs were located interstitially in the short arm, while in the other it was found interstitially in the long arm (Fig. 1c). This result confirms the effectiveness of the hybridization process.

FISH experiments with a 5S rDNA probe, allowed the identification of two chromosome pairs with clusters of these genes, as described by FUJIWARA et al. (1998). Two signals were observed in the short arms of a subtelocentric chromosome pair and other two signals were observed in two submetacentric chromosomes, one in the short arms and the other in the long arms (Fig. 2). The presence of a 5S cluster at an interstitial position in the long arms of a submetacentric chromosome has not been observed in previous studies of this species (FUJIWARA et al. 1998).

To analyze the relative position of genes 18S and 5S in the chromosomes of F_1 individuals, slides with fish metaphases were treated by the FISH technique and 5S probes, photographed (Fig. 2b) and subsequently stained with silver nitrate to identify NORs (Fig. 2a). These experiments allowed us to verify that two submetacentric chromosomes were stained by the two methods indicating that both 18S and the 5S rDNA genes were tandem arranged in these chromosomes, as described by FUJIWARA et al. (1998). Interestingly, one submetacentric chromosome exhibited 18S and 5S genes at an interstitial position in the short arms and the other showed these genes at an interstitial position in the long arms.

The experiment described above allows us to conclude that the difference in position between 18S and 5S was due to a chromosome rearrangement that involved the whole chromosomal segment that contains both gene clusters. Apparently, the rearrangement responsible for this change was a pericentric inversion, but the present data, do not permit to reject the hypothesis that a transposition occurred.

The presence of NORs and 5S genes in the short arms of a submetacentric pair has been described in all rainbow trout stocks examined so far (SCHMID et al. 1982; PHILLIPS and IHSSEN 1985; MAYR et al. 1986; UEDA and KOBAYASHI 1988; LLOYD and THORGAARD 1988). Therefore, the presence of NORs and 5S genes in the long arms of a submetacentric chromosome observed in fish from Campos do Jordão is probably a derived condition, characteristic of the fish used to originate this stock.

Knowing the position of the ribosomal genes and whether they are located in the same chromosomes in different stocks is of great importance for the management and cultivation of rainbow trouts. If more than one chromosome pair is involved, some crosses may yield partially unviable offsprings due to the absence of the major ribosomal genes. Further studies of the meiotic cells of the hybrids may enlighten the maintenance mechanism of the different NOR phenotypes found in the rainbow trout stocks.

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