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Physical distribution of SINE elements in the chromosomes of Atlantic salmon and rainbow trout

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SINE sequences are interspersed throughout virtually all eukaryotic genomes. In the family Salmonidae three families of SINEs have been identified. In this work we have attempted to characterize and locate by means of fluorescent *in situ* hybridization (FISH) one of these families (*Hpa*I) in the genome of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Our results show that these SINEs are interspersed throughout all the chromosome pairs except for the heterochromatin-positive areas, generating a banding pattern that could be useful for chromosome identification in both species.

Keywords: chromosome location, fluorescent *in situ* hybridization, *Hpa*I, *Oncorhynchus mykiss*, *Salmo salar*, SINE.

Introduction

Highly repetitive sequences are interspersed throughout virtually all eukaryotic genomes. They can be classified into two categories based on their size: long interspersed repetitive elements (LINEs) and short interspersed repetitive elements (SINEs). They have been isolated from the genomes of many multicellular organisms from invertebrates to vertebrates (Ohshima *et al*., 1993; Okada & Ohshima, 1995). SINEs are typically less than 500 base pairs in length and can be unique to a taxonomic rank, for example a family, a genus or a few species (Smit, 1996). Almost all SINEs reported to date are derived from tRNA (Okada, 1991a,b; Okada & Ohshima, 1995), with the exception of the primate *Alu* and the rodent B1 families which are derived from 7SL RNA (Ullu & Tschudi, 1984). SINEs are believed to be amplified by a process of retrotransposition (Singer, 1982). In contrast to DNA transposable elements, SINEs appear to be inserted irreversibly and thus they should serve as ideal evolutionary and phylogenetic markers (Okada, 1991b).

In the genome of salmonid fishes both classes of sequences have already been identified (Moir & Dixon, 1988). Kido *et al*. (1991) have characterized three families of SINE sequences derived from tRNA: *Sma*I, *Fok*I and *Hpa*I families. The salmon *Sma*I family is restricted to the genomes of chum salmon (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*). The *Fok*I family is only present in species from the genus *Salvelinus*, and the *Hpa*I family is present in all the species of the salmonid family. The presence of specific elements of the different families has been used to infer phylogenetic relationships among salmonids (Murata *et al*., 1996) and more recently to obtain DNA fingerprint patterns (Spruell & Thorgaard, 1996). In this work, we present the results of our fluorescent *in situ* hybridization (FISH) experiments on the chromosomes of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) using highly repetitive sequences belonging to the *Hpa*I family.

Materials and methods

PCR amplifications

A set of primers based on the *Hpa*-345 locus of Kokanee salmon (*Oncorhynchus nerka adonis*) (Murata *et al*., 1993) was designed for specific amplification of the *Hpa*I family in the genome of Atlantic salmon and rainbow trout. PCR was performed with a Perkin Elmer 2400; the amplification mixture for PCR comprised 5–20 ng of genomic template DNA, 150 pmol of each primer, 200 μM of dNTPs and 1 unit of Taq polymerase (Boehringer). Amplification reactions were performed in a thermal cycler (Perkin Elmer 2400) with 30 cycles consisting of 95°C for 1 min, 58°C or 60°C for 1 min, and 72°C for 1 min.

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Cloning and nucleotide sequence

The PCR amplified products were treated with kinase, electrophoresed, excised from the gel using a silica matrix (Bio 101) and ligated in the S*ma*I site of the pUC18 cloning vector, then sequenced in both strands by using the Sequenase Kit (USB) with universal primers. The reaction products were fractionated by electrophoresis on a 6% polyacrylamide gel. DNA sequences were searched for homologies using the nonredundant amino acid sequence database at the NCBI, with the BLAST program.

Cosmid library

A total genomic library of Atlantic salmon was constructed in a cosmid vector (sCos-1, Stratagene). Cosmids were isolated by plating out the library at low density and selecting after hybridization with the *Hpa*I unit sequence obtained from the rainbow trout PCR amplification. The probe was labelled by a random priming technique using (α^{32}P)dATP (Amersham). Filters were hybridized at 65°C in 3× SSPE, 0.5% dried milk, 1% SDS and 6% polyethylene glycol overnight, and washed in 1× SSC, 0.1% SDS at 65°C. Positive bands isolated from the genomic cosmid were excised, cloned and sequenced in both strands.

Chromosome preparation and FISH

Blood was collected from the dorsal artery of 10 large fish species, anaesthetized with MS-222, using a heparinized syringe. Metaphases were obtained from blood cultures as described by Sanchez *et al*. (1990).

Probes were labelled with biotin-11-dUTP using a nick translation kit (Boehringer). Details of probe preparation, *in situ* hybridization methods and the analysis of metaphases are described in Pendas *et al*. (1993a,b).

Results and discussion

PCR products showed a band of 395 bp in the case of rainbow trout and a band of 255 bp in Atlantic salmon. Cloning and sequencing of four PCR products of each repeat type indicated that rainbow trout contained one unit of the *Hpa*I family, whereas this family was not present in the PCR band resulting from the Atlantic salmon genome amplification. The complete sequences of the two bands are identical to those obtained by Murata *et al*. (1993) after the amplification of Kokanee salmon and brown trout, respectively (data not shown).

With the aim of locating the *Hpa*I family in the genome of Atlantic salmon, a total cosmid genomic library from this species was screened with the *Hpa*I unit sequence obtained from the rainbow trout amplification. Positive clones were selected and digested with different restriction enzymes and blotted onto a membrane filter. The filter was then hybridized with the *Hpa*I unit. Two positive bands resulting after the digestion of one of the positive cosmids with *Ava*II were cloned and sequenced. The larger fragment cloned was 342 bp long (Fig. 1a) and showed 68% homology to the coho salmon (*Oncorhynchus kisutch*) short interspersed element *Hpa*-51 that is also present in the genome of other Pacific salmon species (Murata *et al*., 1993). The smaller band, which was 246 bp long (Fig. 1b), showed 82% identity with the *Salmo salar* transposon like Tc1-encoded transposase pseudogene isolated by Goodier & Davidson (1994).

(a)
seq 342 bp

ACCCACCAGTCAAAAGGGGCACACCTATTCATTCAAGGGTTTTTATTTATTTTTTACAATTTTCTACATTGTAG
AATAATAGTGAAGACAAGAAAACTATGAAATAACACATATGGAATCATCATGTAGTAACCAAAAAAGATCA
ACCAAATCATAATATATTTCATATTTGAGATTCTTCAAAGTAGCCACCCTTTGCCTTGATGAGTTTTGCACAC
ACTTGGCATCTCTCAACCAGCTTCATGAGGTAGTCACCTGGATGCATTTCAARGAACAGGTCTCCGTTGTTAA
AAGTTAATTTGTGGAATTTCTTCCTCTAGCGTGCACTAGTTGTGTGACAGG

(b)
seq 246 bp

CCCACTTTGTTGAAGCCCCTTTGGCAGTGATTACAGCCTGAAGTCTTCTTGGGATGACGCTATAAGTTGGCAC
ACCTGTATTTGGGGAGTTTCTCCCATTCTTCTCTGCAGATCCTCTCAAGGCTGTCAGGTTGGACTGAGTGTCG
CTGCACAGCTATTTTCAGGTCTCCAGAGGATATTCGCTGGTCAAGTCTGGGCTGCTGGGCCCACTCAAGGACA
TTGAGACTTATTCCGAAGCCACTCTGG

Fig. 1 Nucleotide sequence of the two bands cloned from the Atlantic salmon cosmid containing a unit of the *Hpa*I family. Similarities with coho salmon short interspersed element *Hpa*-51 (a) and *Salmo salar* transposon (b) are underlined.

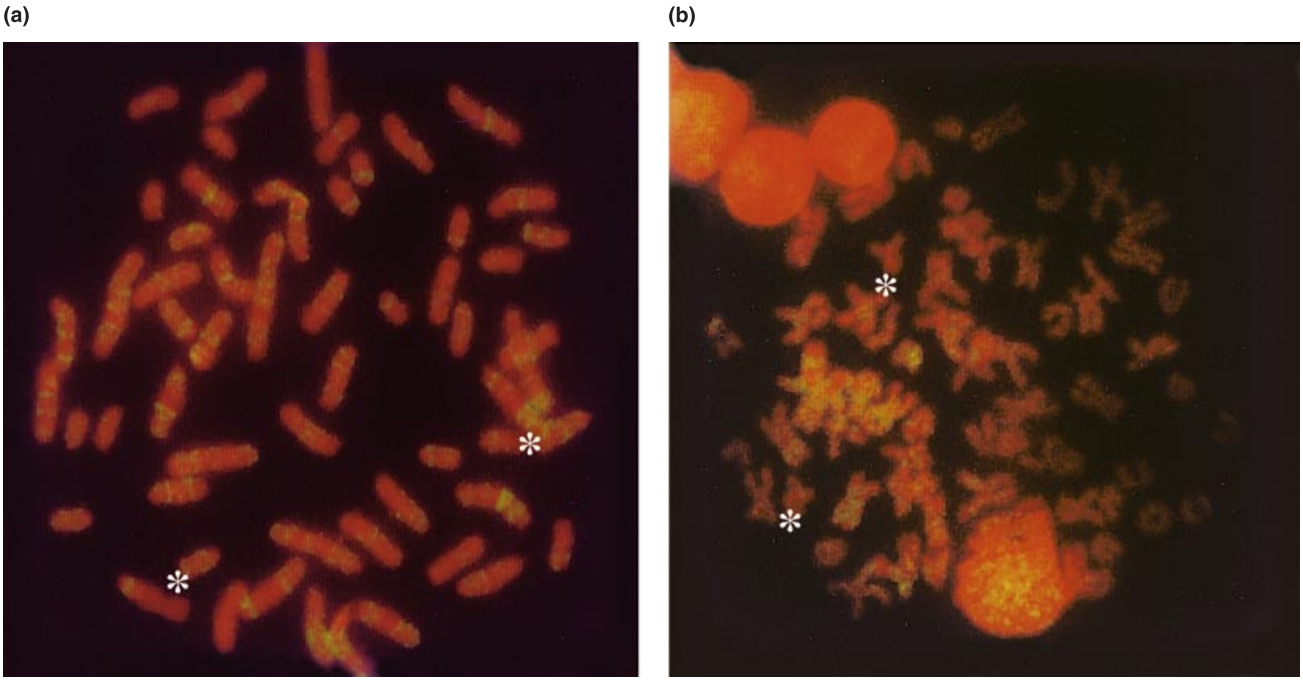

Fig. 2 Fluorescent *in situ* hybridization (FISH) showing (a) Atlantic salmon chromosomes hybridized with a PCR amplified *Hpa*I SINE fragment from rainbow trout. (b) Rainbow trout chromosomes hybridized with the same probe. Asterisks indicate the rDNA-bearing chromosome arms where no hybridization sites were detected.

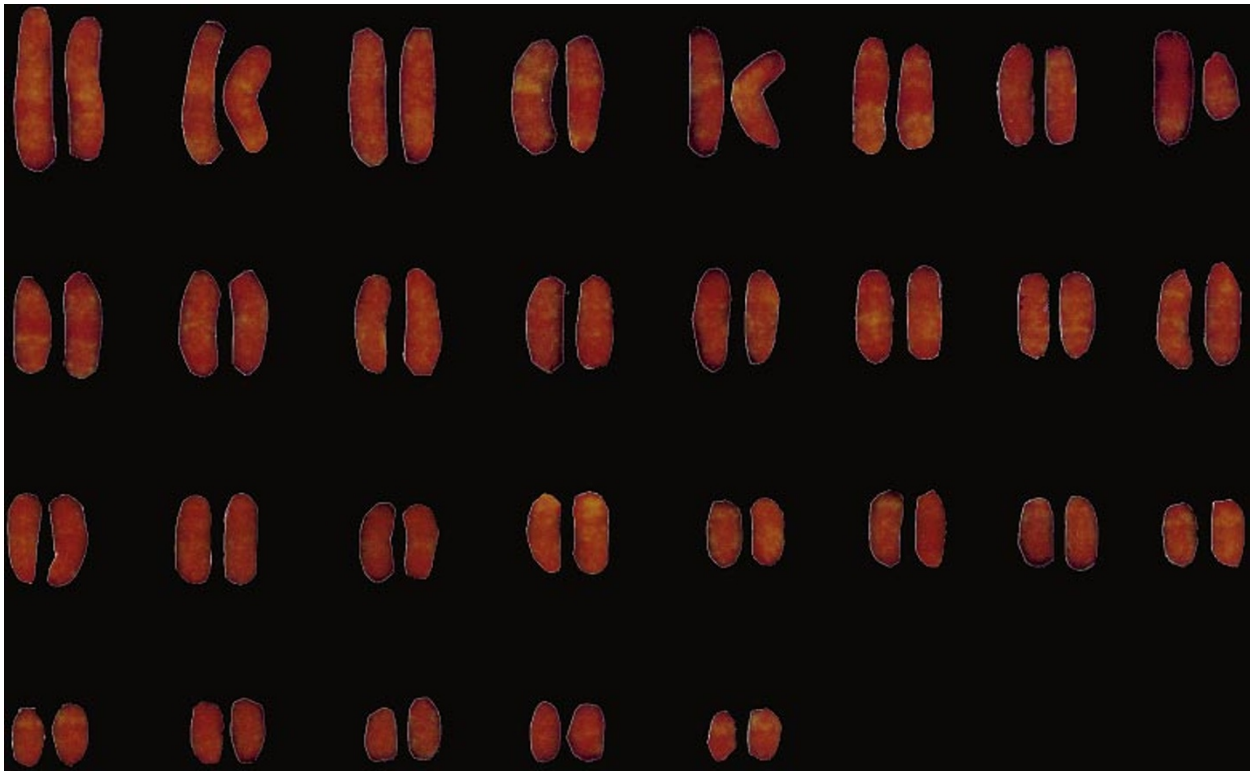

Fig. 3 Homologous pairs identified from the banding pattern of chromosomes shown in Fig. 2(a). This Atlantic salmon karyotype is $2n = 58$.

In order to determine the chromosomal distribution of the *Hpa*I family, FISH analysis was carried out in the two species using as probes the PCR-amplified 395 bp fragment from rainbow trout and the 255 bp fragment from Atlantic salmon, the positive cosmid isolated for the *Hpa*I family and the cloned band from this cosmid that contained a part of the *Hpa*-51 locus. As expected, hybridization of the PCR product from the Atlantic salmon lacking *Hpa*I family members did not generate any signal in either species. The hybridization signals obtained with the other three probes revealed that the *Hpa*I sequence was interspersed throughout all the chromosome pairs except in the heterochromatin positive areas, for both Atlantic salmon and rainbow trout (see Fig. 2), providing hybridization patterns complementary to the C-banding. Hybridization patterns obtained using the sequence similar to the *Hpa*-51 locus were identical to the patterns obtained using the whole genomic cosmid. The absence of the SINE sequence in the heterochromatic chromosome arm where the rDNA is located (Pendas *et al*., 1993a) indicates that the members of this SINE family are located only in the euchromatic regions of the chromosomes.

Nonrandom distribution of SINE elements has been described in human chromosomes. For example, the *Alu* and *L*1 families are preferentially found in R and G bands, respectively (Korenberg & Rykowski, 1988); therefore, the distribution of the SINE element found in our work is not surprising. In addition to this particular distribution, we have observed that the *Hpa*I element contained in the cosmid is located adjacent to a Tc-1 transposon-like element (Goodier & Davidson, 1994). Spruell & Thorgaard (1996) have suggested that these two features of SINEs (nonrandom distribution and integration close to other repetitive elements), together with the occurrence of some SINE elements as tandemly repeated arrays (Nagahashi *et al*., 1991), could be responsible for the production of fingerprinting patterns instead of a smear when these salmon SINE families are used for Southern blot analyses.

The physical distribution of a member of the *Hpa*I family obtained in this work on Atlantic salmon and rainbow trout chromosomes can probably be extended to the other two SINE families (*Fok*I and *Sma*I). If assayed on chromosomes from all the other members of the Salmonidae family, FISH of SINE elements could provide new information useful in evolutionary studies (Murata *et al*., 1993). This method could also contribute to standardizing the Atlantic salmon karyotype, because the fluorescent *in situ* hybridization of these sequences gives a banding pattern that allows the identification of at least 15 chromosome pairs (Fig. 3).

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