Comparative chromosome painting in fish using human sex chromosome probes

Article · January 2003

CITATION
1

READS
24

1 author:

Ashutosh Halder
All India Institute of Medical Sciences
90 PUBLICATIONS **606** CITATIONS

SEE PROFILE

Indian J. Fish., **50**(1) : 103-106, Jan.-Mar., 2003

Comparative chromosome painting in fish using human sex chromosome probes

N.S. NAGPURE, B. KUSHWAHA, SATISH K. SRIVASTAVA, A. HALDER AND A.G. PONNIAH

National Bureau of Fish Genetic Resources, Canal Ring Road, Telibagh, P.O. Dilkusha, Lucknow - 226 002, India

ABSTRACT

The study was undertaken to identify sex chromosomes in *Channa punctatus* using human sex chromosome specific paint probes. Although the probes could detect sex chromosomes in human metaphase spread, no hybridization signals were detected on any of the chromosomes in *C. punctatus*. The results of the present study indicated that human sex chromosome paint probes were not useful for detecting sex chromosomes in *Channa punctatus*.

Introduction

Sex determination is very complex phenomenon in fishes and is determined by genetic/ environmental factors. In genetically sex determined fishes male heterogamety, female heterogamety and multiple sex chromosomes are known. So far, sex chromosomes have been described in few species by karyotyping and chromosome banding, since sex chromosomes are not morphologically differentiated in many fish species. The recently developed fluorescence *in situ* hybridization (FISH) technique has been found promising in identification of sex chromosomes in humans and higher mammals. This technique has also been utilized to identify sex chromosomes in few fishes like platy fish, medaka, tilapia and salmonid species (Phillips, 2001; Phillips and Reed, 1996) Further, FISH can be used for comparative cytogenetic studies to investigate the extent and distribution of conserved DNA segments not only between closely related species but also between distantly related species (Phillips, 2001; Matsuda *et al.*, 1998; Scherthan *et al.*, 1994; Hayes, 1995). Probes of multiple copy genes such as ribosomal DNA (rDNA) sequences have been used to locate the NORs in distantly related species, for example human and mouse ribosomal DNA sequences could locate NORs in fishes (Rab *et al.*, 1996; Fischer *et al.*, 2000; Rossi *et al.*, 2000). Similarly it would be interesting if sex chromosome specific DNA sequences obtained from higher mammals can be utilized for interspecific comparison for homologous sequences and eventual identification of sex chromosome in fishes.

The present study was therefore undertaken with a view to identify sex chromosomes in fish by using sex chromosome specific paint probes from human being. An air breathing teleost, *Channa*

punctatus was taken as model fish for this study since diploid chromosome number is lesser and sex chromosomes have not been so far reported (Manna and Prasad, 1973; Sharma and Agarwal, 1981). Sex chromosomes identified on the basis of comparative FISH, can be utilized for sexing cells from immature or adult fish and also for sexing of sperm (Phillips, 2001).

Materials and methods

Chromosome preparations

Chromosome spreads in *Channa punctatus* (n=10) were prepared from kidney tissue, using colchicine-hypotonic-methanol - acetic acid - air drying technique. Metaphase chromosomes from the normal human male were obtained by using standard lymphocyte culture techniques and chromosome slides were prepared by above-mentioned technique. The chromosome slides to be used for in *situ* hybridization were stored at -20°C. Whole chromosome paint (WCP) probes for human X and Y chromosome, directly labelled with two different fluorophores were procured from Vysis, France, for the present study.

The slides were thawed just before use and treated with 70% acetic acid for 2 minutes, followed by dehydration in series of 70, 90 and 100% ethanol for 3 min. each, at room temperature.

Pretreatment:

The dehydrated air-dried slides were incubated in 1.25% acidic pepsin solution for 20 minutes at 37°C followed by washing in phosphate buffered saline (PBS) and double distilled water. Slides were incubated for 10 minutes at 4°C in freshly prepared precooled solution of paraformaldehyde (0.67%) prepared in PBS. Slides were then removed and washed with PBS followed by distilled water and again dehydrated in series of ethanol as mentioned above.

Hybridization:

In situ hybridization of fish and human chromosome was performed simultaneously according to the method of Pinkel *et al.* (1986) with suitable modifications. In brief 5 µl hybridization cocktail consisting of 3.5 µl hybridization buffer, 0.5µl each of X and Y probe and 0.5µl triple distilled water was applied to each slide, covered with a 24x 32mm coverslip, and sealed with rubber cement. Denaturation of probe and chromosomes was performed simultaneously on a hot plate at 74°C for 5 mins. Slides were incubated overnight in moist chamber at 37°C. The rubber cement and cover slip were removed and the slides were washed by incubating in solution of 0.3% NP-40 (supplied by Vysis Inc. France) at 74 ± 1°C for 3 minutes followed by agitating in solution of NP (0.1%) at room temperature for 2-3 minutes. Slides were then dehydrated in series of ethanol at room temperature. Dried slides were stained and mounted with 10µl DAPI-II with antifade followed by sealing with transparent rubber seal around the edges of coverslip.

Detection and visualization of hybrid probes:

The slides were observed under Nikon fluorescent microscope using orange/green filter. The FISH images were captured by cooled CCD camera and converted to computer image by Cytovision Unix 4.1 software (Applied imaging) for analysis.

Results and discussion

Counterstaining by DAPI facilitated identification of chromosomes of *C. punctatus* and all the specimens were found to possess diploid chromosome

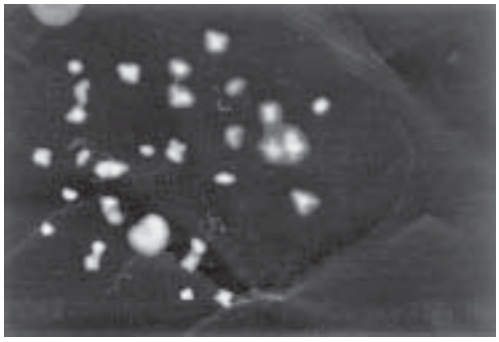

Fig. 1. Metaphase chromosome spread of *Channa punctatus*, without hybridization signals.

number of 2n= 32 (Fig.1) which is consistent with earlier reports (Manna and Prasad, 1973; Sharma and Agarwal, 1981). Fluorescent signals were not observed on any chromosomes of *C. punctatus*, which indicated that hybridization of WCP probes to *C. punctatus* chromosomes could not take place, due to absence of homologous DNA sequences between fish and human sex chromosomes, since both the species are phyllogenetically distant. On the other hand, chromosomes from normal human male, when hybridized with same probe, fluorescent signals were observed in all

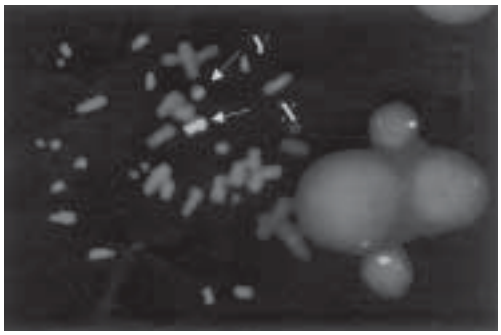

Fig. 2. Metaphase chromosome spread of human male showing X and Y sex chromosome painted with fluorescent probes.

the metaphase spreads (Fig. 2) which served as positive control. X chromosome in human metaphase spread exhibited green signal, while small chromosome with red signal indicated presence of Y chromosome. The FISH signals could be detected even in human interphase cells (Fig. 2) which confirmed the suitability of this technique to characterize sex at cellular level. Further, hybridization of paint probes to human X and Y metaphase chromosomes ensured the correctness of the methodology.

Studies on comparative mapping and NOR variability have been done earlier using mouse and human rDNA sequences as chromosome specific probes in puffer fish *Tetraodon nigroviridis* and *Oedalechilus labeo* (Fischer *et al.*, 2000; Rossi *et al.*, 2000). Similarly, the (TTAGGN) n, sequences found at human telomeres have been found conserved in many vertebrate species (Meyne *et al.*, 1989), hence telomeric probes have also been examined in *Oedalechilus labeo* in which telomeres exhibited strong double signals (Rossi *et al.*, 2000). However, lack of hybridization of human sex chromosome probes to *C. punctatus* chromosomes as observed in the present study indicated that human sex chromosome paint probes were not useful for detecting sex chromosomes in *Channa punctatus.*

Acknowledgement

The authors are grateful to the Head, Department of Medical Genetics, Sanjay Gandhi Post Graduate Medical Institute, Lucknow, for providing laboratory facilities and valuable suggestions.

References

Fisher, C., C. Ozouf-Costaz, H. Roest Crollius, C. Dasilva, O. Jaillon, L. Bouneau, C. Bonilla, J. Weissenbach and A. Bernot 2000. Karyotype and location of characteristic tandem repeats in the puffer fish *Tetraodon nigrovirdis. Cytogenet. Cell Genet.*, **88**: 50-55.

Hayes, H. 1995. Chromosome painting with human chromosome specific DNA libraries reveals the extent and distribution of conserved segments in bovine chromosomes. *Cytogenet. Cell Genet.*, **71**:168-174.

Manna, G.K. and R. Prasad 1973. Chromosomes in three fish (*Channa*). *Nucleus*, **16**(3): 150-157.

Meyne, J., R.L. Ratliff and R.K. Moysis 1989. Conservation of human telomere sequence (TTAGGG)n among vertebrates. *Proc. Nat. Acad Sci. USA*, **86**:7049-7053.

Matsuda, M., C. Matsuda, S. Hamaguchi and S. Sakaizumi 1998 Identification of sex chromosomes of the medaka, *Oryziae latipes*, by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.*, **82**: 257-262.

Phillips, R.B. and K. Reed 1996. Application of fluorescent *in-situ* hybridization (FISH) to fish genetics: a review. *Aquaculture*, **143**(3): 197-216.

Phillips, R.B. 2001. Application of fluorescent *in-situ* hybridization (FISH) to fish genetics and genome mapping. *Mar. Biotechnol.*, **3**: S141-S152.

Pinkel, D., T. Straume and T.J.W. Gray 1986. Cytogenetic analysis using quantitative, high sensitivity fluorescence hybridization. *Proc. Nat. Acad Sci. USA*, **83**: 2934-2938.

Rab P., K.M. Reed, Abel Ponce de Leon and R.B. Phillips 1996. A new method for detecting nucleolus organizer regions in fish chromosomes using denaturation and propidium iodide staining. *Biotechnic and Histochemistry*, **71**(3): 157-162.

Rossi, A.R., E. Gornung, Crosetti, De Innocentiis and L. Sola 2000. Cytogenetic analysis of *Oedalechilus labeo* (Pisces: Mugilidae), with a report of NOR variability. *Marine Biology*, **136**:159-162.

Sharma O.P. and A. Agarwal 1981. Cytogenetic studies on two species of genus *Channa*. *Pers. Cyt. Genet.*, **3**: 57-61

Scherthan, H., T. Cremer, U. Arnason, H.U. Weier, A. Lima-de-Faria, L. Fronike 1994. Comparative chromosome painting discloses homologous segments in distantly related mammals *Nat. Gent.*, **6**: 342-347.