Dear Editor:

Fish cell lines have applications in numerous investigation fields: biomedical research, toxicology, virology, basic fish research, etc. (7,12). Because the possibility of genetic analysis of the somatic cells is potentially of great interest, it seemed useful to study continuous fish cell lines in order to better know the chromosomal variations occurring in vitro. Furthermore, the application of improved banding techniques to the chromosomes of fish cell lines is very useful in the identification of chromosomal rearrangements.

We have initiated a cytogenetical research with salmonid cell lines as matter of study (3,15). In this paper, we report the chromosomal analysis of two salmonid fish cell lines named CHSE-214 (Oncorhynchus tschawytscha) (8) and RTG-2 (Oncorhynchus mykiss) (17). CHSE-214 cell line was derived from chinook salmon (Oncorhynchus tschawytscha) embryo (8) and the RTG-2 cell line was derived from rainbow trout (Oncorhynchus mykiss) gonadal cells (17). Both are monolayer cultures of fibroblastlike cells. The procedures used for maintenance and for chromosomal analysis were described in an earlier publication (15).

Chromosome numbers of CHSE-214 and RTG-2 were determined by conventional Giemsa staining. Both cell lines showed variations in this characteristic, the modal chromosome number was 64 for CHSE-214 (O. tschawytscha, 2n = 68) and 55 for RTG-2 (O. mykiss, 2n = 60). The reduction in the chromosome number observed in CHSE-214 and RTG-2 cell lines can originate by a loss of chromosomal units and also by chromosomal rearrangements. One long submetacentric chromosome that is not described in the karyotype of the original species appeared persistently in each cell line (95% and 80% of metaphases examined, from CHSE-214 and RTG-2, respectively). These two abnormal chromosomes are designated as marker chromosomes CI and RI, from CHSE-214 and RTG-2, respectively (Figs. 1 and 2).

C-banding revealed that constitutive heterochromatin was localized at the centromeres and telomeres of all the chromosomes of the two cell lines, and was also associated with the NOR regions. Interstitial C-bands appeared in some large and medium acrocentric chromosomes of CHSE-214 cell line. CI and RI marker chromosomes showed interstitial C-bands (Fig. 2).

The digestion with AluI, DdeI, HaeIII, and HinfI REs produced reproducible and well-defined banding patterns on CHSE-214 and RTG-2 chromosomes (data not shown). The pattern obtained in CHSE-214 cell line with AluI restriction endonuclease was particularly different from that produced by all the enzymes in CHSE-214 or RTG-2 chromosomes. This pattern shows large intercalar heterochromatic regions of CI marker and two large acrocentric chromosomes (named “exceptional” chromosomes) to be completely digested. Through the analysis of response to in situ digestion with these REs two different types of heterochromatin could be identified in CHSE-214 and RTG-2 chromosomes: (a) heterochromatic regions always resistant to all enzymes assayed. These regions included the telomeric areas of all chromosomes and the interstitial heterochromatin and (b) intercalar heterochromatin of CI marker and the “exceptional” CHSE-214 chromosomes, centric heterochromatin and NOR-associated heterochromatin, which showed variable behavior depending on the RE used.

After Bromodeoxyuridine (BrdU) incorporation and staining of metaphase chromosomes by the fluorochrome-plus-Giemsa (FPG) technique, a replication banding pattern could be demonstrated along chromosomes of both cell lines (Fig. 1 b and d). In this pattern, dark areas corresponded to DNA replicated before BrdU incorporation (early replicated DNA) and light ones corresponded to late replicated DNA. Centromeric regions of metacentric chromosomes, interstitial regions, and NOR-regions exhibited pale staining. This fact indicates that these regions, as heterochromatic regions, replicated entirely during the late S-period. The existence of this banding pattern in the chromosome of a fish cell line (3,15) and fish chromosomes (1,4,6) have been cited as evidence of the replication in fish, similar to those of higher vertebrates, are made up of small alternating early- and late-replicating segments. The successful obtention of replication bands in fish chromosomes has the practical value of producing more detailed banding patterns as an aid to more accurate karyotypic analysis. Furthermore, replication banding patterns can provide the clearest evidence for homologies among chromosomes of different species.

The combined analysis of the banding patterns obtained by means of C-, RE, and Replication banding on marker chromosomes CI and RI have permitted us to identify the possible origin of CHSE-214 and RTG-2 marker chromosomes. Phillips et al. (14) have described the existence of several acrocentric chromosome pairs in the karyotype of O. tschawytscha (the original species of CHSE-214 cell line) with large interstitial heterochromatin bands, by means C- and Quinacrine staining. However, CHSE-214 cell line showed only three chromosomes with that banding pattern: CI marker and the “exceptional” CHSE-214 chromosomes. The size, morphology, and banding patterns of one of the “exceptional” chromosomes indicate that this one is, probably, the homologous of one of the two chromosomes engaged in the origin of CI marker chromosome. Consequently, it is probable that CI marker arose from a centric fusion of two acrocentric chromosomes nonhomologous (Robertsonian fusion). The homologous chromosome of the remaining “exceptional” chromosome could be lost following the evolution of CHSE-214 cell line or could be involved in other kinds of chromosomal rearrangements.

With regard to RI chromosome marker, this chromosome presented an intercalar positive C-band, resistant to all the REs tested in this study (Fig. 2). There is not any chromosome from RTG-2 original species (O. mykiss) that showed any intercalar heterochromatic region (9). According to this, we suggested that RI marker chromosome was
originated by the junction between the centromeric region of an acrocentric chromosome and the telomeric end of a metacentric chromosome (Tandem fusion). This study and other ones including cytogenetic characterization of the Atlantic salmon cell line (3,15), point to Robertsonian and tandem fusions as the more common mechanisms involved in the chromosomal evolution of salmonid cell lines.

All Ag-stained metaphases of both cell lines showed two NOR-bearing chromosomes. The morphology of these chromosomes revealed that they are not homologous (Fig. 1 a and c). In CHSE-214, NOR-region was located at the telomere of a small acrocentric chromosome and in the short arm of a medium acrocentric chromosome, while the telomeric regions of two different metacentric chromosomes showed to be the NOR-bearing chromosomes in RTG-2 cell line.

The study of NOR-regions in CHSE-214 and RTG-2 chromosomes by Ag-staining revealed other specific property of CHSE-214 and RTG-2 cell lines. In both cell lines, the size and morphology of one of the two NOR-bearing chromosomes agree with the size and morphology described for the NOR-bearing pair of the original species (13,16). The remaining NOR appeared in a different chromosome of the two fish cell lines karyotypes. No multichromosomal location of NOR-genes have been described for O. tshawytscha and O. mykiss (the original species of CHSE-214 and RTG-2 cell lines, respectively), at least with the conventional techniques (10,14).