Nucleolar dominance in interspecific hybrids of cyprinid fishes

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Abstract

The occurrence of nucleolar dominance was documented cytologically in reciprocal F_1 hybrids between two species of cyprinid fishes. Both the golden shiner, *Notemigonus crysoleucas*, and rudd, *Scardinus erythrophthalmus*, possess 2n = 50 chromosomes and a single pair of medium-sized acrocentric chromosomes bearing the nucleolus organizer region (NOR) on the short arm. Over 50% of the hybrids from both reciprocal crosses exhibited only a single silver-stained NOR chromosome in all metaphases examined; the remainder exhibited only a single silver-stained NOR chromosome in about 75% of the metaphases examined. All individuals surveyed from both parental species exhibited two silver-stained NOR chromosomes in all metaphases examined. G-band patterns of the NOR chromosomes of the two species were found to differ and were used to identify the single silver-stained NOR chromosome in the F_1 hybrids. In each case, the single NOR chromosome was found to be that of the maternal species, suggesting a maternal or cytoplasmic effect to the nucleolar dominance.

Introduction

Nucleolar dominance, the phenomenon where cells of interspecific hybrids primarily or exclusively express the ribosomal RNA (rRNA) genes of the nucleolar organizer region (NOR) of only one of the two parental species, is well known in several genera of plants (Wallace and Langridge, 1971; Flavell and Martini, 1982; Reeder, 1985), the frog genus *Xenopus* (Honjo and Reeder, 1973; Cassidy and Blackler, 1974; Reeder and Roan, 1984), *Drosophila* (Durica and Krider, 1977; Bicudo and Richardson, 1977), and races of the *Steropleurus martorelli* (Orthoptera) complex (Santos *et al.*, 1989). This phenomenon has also been reported in mouse-human somatic cell hybrids (Miller D. *et al.*, 1976; Miller O. *et al.*, 1976; Onishi *et al.*, 1984). In most cases, the transcriptional dominance of the NOR of one of the parental species has either been assumed or been shown to be complete and to be the same in reciprocal crosses (Bicudo and Richardson, 1977; Onishi *et al.*, 1984; Reeder, 1985).

There is evidence, however, that the dominance is not always complete and that the dominance effect lessens with increasing developmental age (Honjo and Reeder, 1973; Cassidy and Blackler, 1974). The mechanism(s) behind the phenomenon are not well known (Hadjiolov, 1985), although at least two distinct molecular explanations have been proposed (Reeder, 1985). Nucleolus activity, *i.e.* rRNA gene transcription, is probably regulated by several factors, including the rRNA genes themselves, associated nuclear gene interactions, developmental stage and tissue specificity (Honjo and Reeder, 1973; Cassidy and Blackler, 1974; Miller O. *et al.*, 1976; Flavell and Martini, 1982; Reeder, 1985). There also

appears to be a maternal or cytoplasmic effect, at least during early developmental stages (Honjo and Reeder, 1973; Cassidy and Blackler, 1974; Bicudo and Richardson, 1977). In general, nucleolar dominance appears to be a consequence of evolutionary divergence of either the rRNA genes, their transcriptional regulation, or both (Wallace and Langridge, 1971; Reeder, 1985).

Our interest in nucleolar dominance in cyprinid fishes stemmed from earlier work (summarized in Amemiya and Gold, 1990; Amemiya *et al.*, 1991) on the chromosomal locations of NORs among North American cyprinid species. The primary purpose of those studies was to utilize chromosomal NORs as systematic or taxonomic markers. During the course of these studies, we found that approximately 10% of all individuals examined were heterozygous for one of two types of intraspecific NOR heteromorphisms. These included: (1) consistent differences between chromosomally homologous NOR sites in the size of silver-stained NORs; and (2) the absence of silver-stainability at one of two chromosomally homologous NOR sites. Since the silver-stainability of a given NOR is thought to represent an indication of its transcriptional activity at the preceding interphase (Miller D. *et al.*, 1976; Howell, 1977, 1982; Hubbell, 1985), the heteromorphic phenotypes were similar to those expected (and observed) under nucleolar dominance (Miller D. *et al.*, 1976; Miller O. *et al.*, 1976; Santos *et al.*, 1989).

In this paper, we document cytologically the occurrence of nucleolar dominance in F_1 hybrids between two species of cyprinid fishes. To our knowledge, this is the first report of nucleolar dominance in fish.

Materials and methods

The species used in this study were the golden shiner, *Notemigonus crysoleucas*, and the rudd, *Scardinus erythrophthalmus*. The former is endemic to North America and is thought to belong to the cyprinid subfamily Abramidinae (Miller, 1959); the latter is endemic to Europe and is thought to belong to the cyprinid subfamily Leuciscinae (W. Rainboth, personal communication).

Reciprocal crosses between the parental species (*i.e.*, female golden shiner x male rudd, and female rudd x male golden shiner) were carried out in the spring of 1989 at the Joe Hogan State Fish Hatchery, Arkansas Game and Fish Commission, at Lonoke, Arkansas. Individuals of both species were obtained from the Anderson Minnow Farms in Lonoke, transported to the hatchery, and maintained in separate ponds. Between April 3—6, 1989, two 0.4-ha ponds at the hatchery were each stocked with 340 rudd females which averaged 27 g and with 220 golden shiner males which averaged 41 g. Between April 28 and May 10, 1989, two additional 0.4-ha ponds were each stocked with 220 golden shiner females which averaged 41 g and with 340 rudd males which averaged 27 g. Spawning mats were placed in the ponds, and eggs were allowed to hatch. The F_1 hybrids were then grown in the ponds using standard commercial baitfish culture practices (Giudice *et al.*, 1981). Individuals of the parental species and the F_1 hybrids were obtained by seining the ponds in the spring of 1990 and shipped live to Texas A and M University.

The maternal parent of all F_1 hybrids was verified by comparison of restriction fragments of the mitochondrial DNA (mtDNA) of each F_1 individual with restriction fragments of the mtDNA of individuals from the parental species. Genomic DNA was isolated from selected tissues (primarily heart and muscle) and digested with various type II restriction endonucleases. Restriction fragments were separated in 0.8% agarose gels, transferred to nylon filters according to Southern (1975), and hybridized to a probe labelled with [^{32}P]dCTP by nick translation (after Rigby *et al.*, 1977). Hybridization conditions, washes and autoradiography followed that described by Davis (1986). The probe used was the entire mtDNA molecule of the cyprinid *Cyprinella lutrensis* cloned in lambda bacteriophage, using EMBL arms. The origin of the maternal parent in the F_1 hybrids was easily ascertained from observation of mtDNA fragment patterns digested with the restriction enzymes *Nco*I and *Nhe*I (Figure 1).

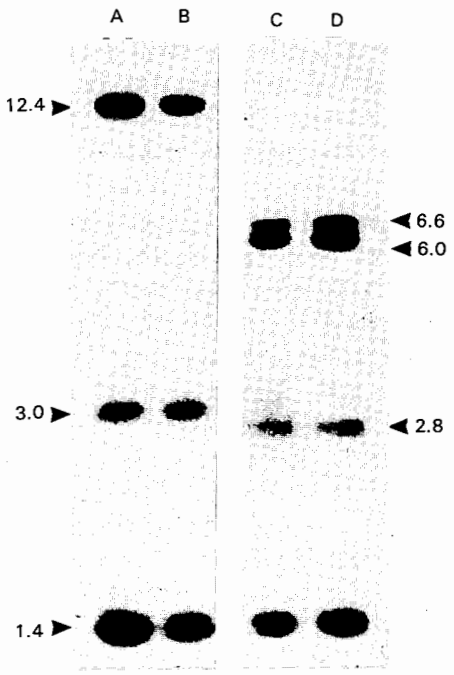

Figure 1 Autoradiogram of single digestions of mtDNA using the enzyme *Nhe*I. Lane A, golden shiner; lane B, F_1 hybrid from cross female golden shiner x male rudd; lane C, F_1 hybrid from cross female rudd x male golden shiner; lane D, rudd. Sizes are in kilobase (kb) pairs.

Metaphase chromosomes from both F_1 hybrids and individuals of the parental species were prepared using both short- and long-term cell cultures as described in Gold *et al.* (1990). Cells were fixed following a procedure outlined in Islam and Levan (1987) and in Gold *et al.* (1990), and microscope slides were prepared as described by Kligerman and Bloom (1977). Metaphases from seven individuals of each of the parental species and from fifteen individuals from each of the reciprocal, interspecific crosses were silver-stained (after Howell and Black, 1980) to resolve chromosomal NORs. G-banding patterns of the NOR-bearing chromosomes were determined using the trypsin-digestion procedure outlined in Gold *et al.* (1990). The latter involved first obtaining (and photographing) G-banded patterns of individual metaphases, followed by destaining the slides in freshly prepared 3:1 (methanol:acetic acid) fixative and subsequent silver-staining. Bright field photomicroscopy followed procedures given in Gold and Amemiya (1986).

Results and discussion

NOR-banding of the golden shiner was previously carried out by Gold (1984). The species possesses 2n = 50 chromosomes and a single pair of NOR-bearing chromosomes, with the NOR located terminally on the short arm of a medium-sized acrocentric chromosome. A silver-stained metaphase from the rudd is shown in Figure 2. This species also possesses 2n = 50 chromosomes and a single pair of NOR-bearing chromosomes with the NOR located on the short arm of a medium-sized acrocentric chromosome. The G-band pattern of the NOR chromosomes in the two species differs: the NOR chromosome of the golden shiner possesses two dark G-bands on the long arm, whereas the NOR chromosome of the rudd possesses three dark G-bands on the long arm (Figure 3). In addition, the NOR chromosome in the rudd is slightly longer than the NOR chromosome in the golden shiner.

Seven individuals from each of the two parental species were screened for silver-stained chromosomal NORs. Nearly all metaphases from all fourteen individuals possessed two silver-stained NORs, indicating that both NOR sites were actively transcribed at a preceding interphase. Among the F_1 hybrids, nine of fifteen individuals examined from the female shiner x male rudd cross, and eight of fifteen individuals examined from the female rudd x male shiner cross, possessed only a single silver-stained NOR in all metaphases examined. G-banding demonstrated that the single, silver-stained NOR in each case was that of the maternal species (Figure 3). Metaphases from the remaining F_1 hybrids (*i.e.* six individuals from the female shiner x male rudd cross and seven individuals from the female rudd x male shiner cross) were mixed, with the majority containing only a single silver-stained NOR (Table 1). All F_1 individuals possessed 2n = 50 chromosomes, suggesting that both NOR-bearing chromosomes (one derived from each species) were present. An example of a G-banded metaphase from an F_1 hybrid containing two silver-stained NORs is shown in Figure 4. The NOR chromosomes from each species can be identified both by G-band pattern and by size.

Table 1 Summary data of reciprocal F_1 hybrids showing one or two silver-stained NOR chromosomes in individual metaphases

Individuals	Number of metaphases examined	Number and percentage of metaphases with: One NOR: No.	%	Two NORs: No.	%
(A) Female golden shiner x male rudd					
1	18	15	83	3	17
2	13	10	77	3	23
3	18	16	89	2	11
4	38	25	66	13	34
5	21	15	71	6	29
6	10	8	80	2	20
Total	118	89	75	29	25
(B) Female rudd x male golden shiner					
1	2	1	50	1	50
2	10	9	90	1	10
3	9	8	89	1	11
4	22	14	64	8	36
5	8	6	75	2	25
6	5	4	80	1	20
7	11	7	64	4	36
Total	67	49	73	18	27

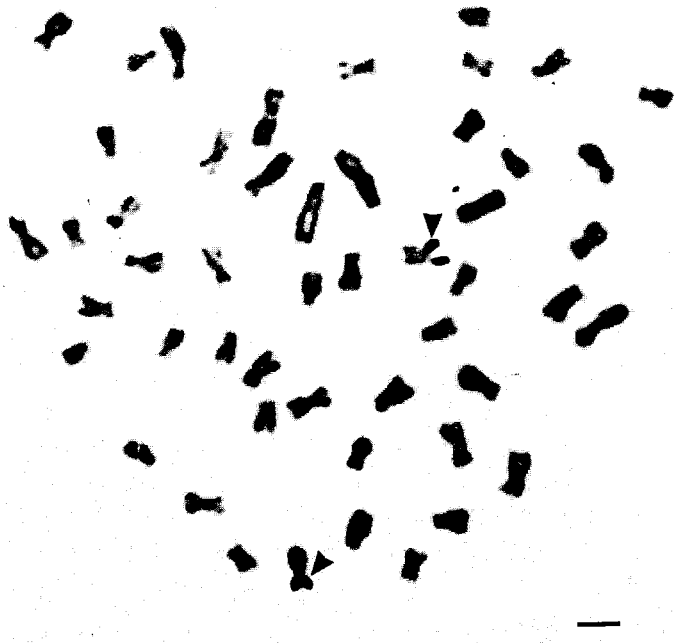

Figure 2 Silver-stained metaphase from rudd. Chromosomal NORs are indicated by arrowheads. Bar scale is the equivalent of 5 μm.

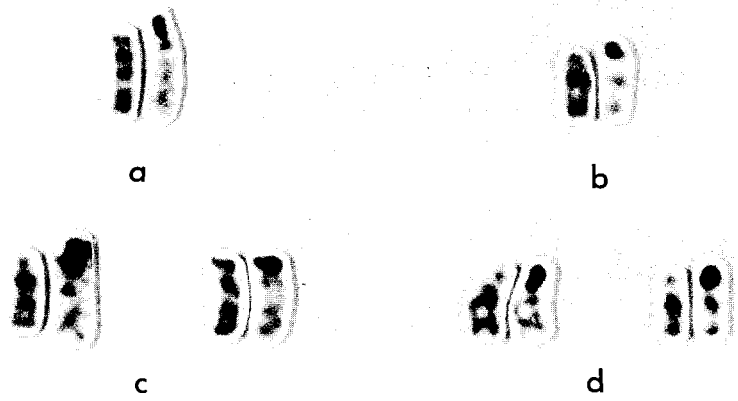

Figure 3 Trypsin G-banded NOR chromosomes: for each pair, the chromosome on the left is from a G-banded metaphase; the chromosome on the right is from sequential silver-staining (to identify NOR chromosomes) from the same metaphase. (a), Rudd; (b), golden shiner; (c), two F_1 individuals from the cross female rudd x male golden shiner; (d), two F_1 individuals from the cross female golden shiner x male rudd.

These results clearly indicate the occurrence of nucleolar dominance in the F_1 hybrids. Over 50% of the F_1 hybrids in both reciprocal crosses exhibited only a single silver-stained NOR, in contrast to individuals from the parental species where two silver-stained NOR chromosomes were observed in all metaphases examined. These findings suggest that rRNA transcription in these F_1 hybrids occurred at only one of the two potential NOR sites. The latter was inferred primarily from the observation that all F_1 hybrids possessed $2n = 50$ chromosomes, the same as both parental species; *i.e.* all F_1 individuals were presumed to possess two potentially functional NOR sites. The remaining F_1 hybrids possessed only one silver-stained NOR site in the majority of their metaphases examined, indicating that the dominance effect was not complete. Similar patterns have been observed in interspecific hybrids of the frog genus *Xenopus*, where the nucleolar dominance effect appeared to decrease with the developmental age of hybrid individuals (Honjo and Reeder, 1973; Cassidy and Blackler, 1974). In our study, the data were primarily from cells taken from differentiated tissues of adult individuals.

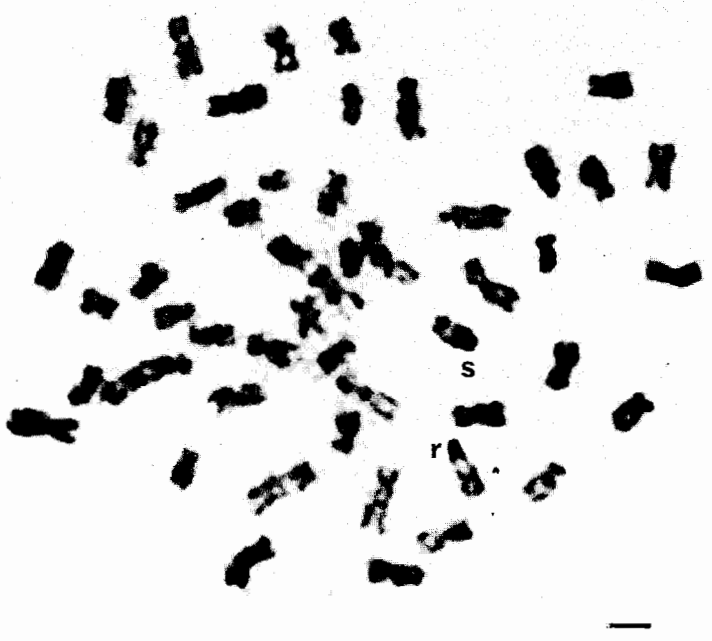

Figure 4 Trypsin G-banded metaphase of F_1 hybrid from the cross female rudd x male golden shiner where both NORs were identified by sequential silver-staining. Rudd NOR chromosome is denoted by r; golden shiner chromosome is denoted by s. Bar scale is the equivalent of 5 μm.

The pattern of nucleolar dominance in the cyprinids differs from that observed in *Xenopus* and *Drosophila*. In the latter, the dominance effect of one of the NOR chromosomes was expressed in genotypically normal F_1 hybrids, regardless of the maternal parent (Honjo and Reeder, 1973; Cassidy and Blackler, 1974; Bicudo and Richardson, 1977; Durica and Krider, 1977). In the cyprinid hybrids, G-band patterns of the silver-stained NOR chromosome indicated that the dominant NOR chromosome was that of the maternal parent. This tentatively suggests a strong maternal or cytoplasmic effect relative to the activity of rRNA transcription and nucleolus expression. However, it should be noted that the evidence for the maternal effect in the F_1 hybrids is based on G-band patterns of two similarly-sized NOR chromosomes. Differences in chromosome contraction are known to affect the number of observed G-bands (Bickmore and Sumner, 1989), so our identification of the single silver-stained NOR chromosome in the hybrids must be considered tentative.

Further studies to identify the rRNAs transcribed in the hybrids are now planned. Nonetheless, the discovery of nucleolar dominance in the F_1 hybrids does appear real and is, to our knowledge, the first reported instance of this phenomenon in fishes.

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