105. Some Features on the Nucleolus Organizer Regions in the Chromosomes of the Cyprinid Fishes

A Preliminary Report

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Nucleolus organizer regions (NORs) which were the chromosomal sites of 18s+28s rRNA genes, can be selectively stained by using the silver staining technique (Goodpasture and Bloom, 1975). This Technique has allowed the demonstration of the chromosomal distributions of NORs in a wide variety of animals and plants. Also in fishes there were several reports on the silver stained NORs by Kligerman and Bloom (1977), Foresti *et al.* (1981), Takai and Ojima (1982), and so on. The present study deals with some features on the NORs revealed by the silver staining technique in the chromosomes of ten species of the Cyprinidae.

Materials and methods. Ten species of the Cyprinidae, collected from the rivers in Kobe City and the Lake Biwa, were used as materials for this study (Table I). The chromosome preparations were made from directly prepared kidney cells according to the conventional air-drying method with Giemsa staining (Ojima, 1982). After the microscopic observation, the slides were destained with 70% alcohol. The silver staining of NORs was carried out following the Ag-AS technique described by Bloom and Goodpasture (1975).

Table I. Results in the analyses of the conventional and silver staining chromosomes

Species	C.N.	Karyotype	No. of NORs
<i>Tribolodon hakonensis</i>	50	14M+26SM+10A	4
<i>Moroco jouyi</i>	50	14M+26SM+10A	4
<i>Zacco temminckii</i>	48	12M+22SM+14A	8
<i>Zacco platypus</i>	48	18M+22SM+8A	4
<i>Opsariichthys uncirostris</i>	78	4M+4SM+70A	2
<i>Ischikauia steenackeri</i>	48	10M+38SM	6
<i>Pseudogobio esocinus</i>	50	16M+28SM+6A	2
<i>Sarcocheilichthys variegatus</i>	50	12M+30SM+8A	4
<i>Gnathopogon elongatus caerulescens</i>	50	12M+26SM+12A	2
<i>Rhodeus ocellatus ocellatus</i>	48	8M+10SM+10A	2

M, metacentrics; SM, submetacentrics; A, acrocentrics.

Figs. 1-10. 1-6: Conventional and silver staining karyotypes in *Tribolodon hakonensis* (1), *Moroco jouyi* (2), *Zacco temminckii* (3), *Zacco platypus* (4), *Opsariichthys uncirostris* (5), and *Ischikauia steenackeri* (6). Arrows show the silver stained NORs (Ag-NORs). Bars indicate inactive NORs without silver stainability. 7-10: Conventional and silver staining karyotypes in *Pseudogobio esocinus* (7), *Sarcocheilichthys variegatus* (8), *Gnathopogon elongatus caeruleus* (9), *Rhodeus ocellatus ocellatus* (10). Arrows show the Ag-NORs.

Results and discussion. The chromosome number and the karyotype constitution in each species were shown in Table I.

After the silver staining, a pair of homologous chromosomes in four species, two pairs of ones in four species, three pairs of ones in one species, and four pairs of ones in one species, revealed the silver stained NORs (Ag-NORs) commonly at the terminal regions of the short arms. The chromosomal sites of NORs thus detected showed a frequent occurrence of faintly stained the secondary constrictions (SCs) in the conventional staining method (Figs. 1–10, Table I).

Morphologically the Ag-NORs of *Rhodeus ocellatus ocellatus* were larger than those of the other species found as the small dots. Also the conspicuous heteromorphism of Ag-NORs between the homologous members was often observed in *R. o. ocellatus* with large NORs. These size differences of the Ag-NORs were closely related to the features of SCs. The SCs of *R. o. ocellatus* with large Ag-NORs showed a distinct appearance corresponding to the Ag-NOR size. The SCs in the other species, however, were not so clear for their small size. Therefore, the detection of the NORs by the conventional staining method prior to that of the silver staining technique was often difficult. Generally speaking, the rDNA content, size of SCs, and silver staining intensity were keenly correlated (Warburton and Henderson, 1979). These size differences found in the Ag-NORs and SCs would be due to the difference of rDNA contents.

In the species carrying NORs on the plural chromosome pairs, the intra-individual variation of the Ag-NOR number was observed. It has been the well known fact that only active NORs are stained with silver. Also, it was difficult to explain the occurrence of the variation by the lack of NORs. These findings indicated that the variation of the Ag-NOR number appeared by the existences of inactive NORs without silver stainability.

So far as NORs have been studied, most fishes have small NORs in only one pair of the specific chromosomes. This seems to be usual and the fundamental condition of the fish NORs. In a very general sense, the ribosomal gene number seems to be correlated with the genome size throughout the evolution (Birnstiel *et al.*, 1971). The fish genome size corresponded to 20–40% of the human genome size. These findings suggested that fishes with the small amount of DNA had a small amount of rDNA and as a result the NORs of fishes showed small size. On the other hand, the large NORs as shown in *R. o. ocellatus* of this study have been known in some species of Gymnotiformes (Foresti *et al.*, 1981), the carp and the funa (Takai and Ojima, 1982), and several cyprinid rhodeine species (Takai and Ojima, unpublished). These large NORs would appear as a result of

an increase of rDNA contents by the translocation, duplication or some other mechanisms. Also, the multiple locations of NORs seem to have been induced from the fundamental condition by the partial translocation of NORs or other mechanisms.

The NORs of the cyprinid fishes showed a very diversified occurrences such as the multiple location, large NORs, and so on. In the other groups, however, the NORs rarely showed such a diversified occurrence. The diversity in the NORs of cyprinids may have occurred closely related to that of the karyotype differentiation in this family.

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