64. Cytogenetic Studies of Hynobiidae (Urodela). II

Banding Karyotype of Hynobius tokyoensis Tago

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Introduction. The chromosome constitutions of Hynobius tokyoensis Tago and other species have been studied by the conventional Giemsa staining method.1-3 For the advancement of the karyotaxonomic studies of the Hynobiidae, more detailed information on the chromosomes at the banding level seemed to be necessary and important. However, in spite of the efforts of a number of investigators,4-13 the detailed chromosome analysis by banding methods has not been so successful except for the identification of the sex chromosomes in Rana4-5 and Triturus.6

We would like to report here the results of the chromosome analysis in Hynobius tokyoensis Tago, making use of the banding patterns obtained by the C-banding treatment, which should provide a possibility of further detailed analysis of the chromosomes of the genus Hynobius.

Materials and methods. Eleven, 5 and 3 embryos of Hynobius tokyoensis Tago from Yokose (Saitama), Yokosuka (Kanagawa), and Chosei (Chiba), respectively, were employed for the chromosome banding analysis. The chromosome preparations of the embryonic cells were performed by the technique previously described by Ikebe and Kohno (1979a),1 using air-drying instead of flame-drying. C-banding slides were made according to the method by Sumner (1972)14 with slight modifications. The slides were stored for 2 or 3 days at the room temperature. Then, they were dipped in 0.2 N HCl for 15–30 min at the room temperature. After rinsing in distilled water, they were incubated for 8–15 min at 50°C in the saturated Ba(OH)₂ solution. Slides were incubated again for 60 min at 60°C in 2×SSC solution and were stained with the 2.5% Giemsa solution (pH 6.8) for about 1.5–2 hr.

Results. An example of the banded metaphases of Hynobius tokyoensis Tago is shown in Fig. 1. Banding analysis of the chromo-

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somes were made in 205, 17 and 6 metaphases in 11, 5 and 3 embryos from Yokose, Yokosuka and Chosei, respectively. The banded partial karyotypes from the above-mentioned two localities (Yokose and Yokosuka) were shown together with the conventional Giemsa stained chromosomes and the diagrams (Fig. 2). Seventeen out of 28 pairs of the chromosomes of *Hynobius tokyoensis* Tago could be identified by this banding method. They were all of the large-sized (nos. 1–9) and the medium-sized (nos. 10–13) chromosome pairs, and four of the small-sized pairs (nos. 14, 15, 21 and 22), according to the chromosome number previously described by Ikebe and Kohno (1979a). As shown in Fig. 2, the bands on the arms of each chromosome formed a distinct pattern, which made it possible to identify each of those chromosome pairs with ease. The remaining 11 of the small sized chromosome pairs showed no characteristic banding patterns.

With the conventional Giemsa staining method, no. 4 and no. 5 chromosomes were very similar and difficult to be distinguished. On banded chromosomes, however, they could easily be discriminated, because no. 4 chromosome had three definite bands on its long arm and no. 5 had two bands (Fig. 2). This banding technique has

Fig. 1. A banded metaphase from an embryonic cell of *Hynobius tokyoensis* Tago, according to the C-banding treatment.
also made the identification possible on nos. 6, 7 and 9 metacentric chromosomes. Although the banding patterns of nos. 6 and 7 were
similar, no. 6 could be identified for the larger band in the short arm, and two bands in the distal region of the long arm. The two bands on the long arm of no. 6 were characteristic as compared with one definite band in the same region of no. 7. No. 9 was slightly smaller than the others and had two large bands in its short arm. The two bands in the short arm of no. 9 were located too close, therefore they looked like a single large band. These bands occupied about two third of the short arm and were easy to be identified. This banding pattern has also made it possible to identify three medium-sized submetacentric chromosomes (nos. 10, 11 and 12). No. 10 chromosome was characterized by a band occupying its whole short arm. No. 11 had three weak bands in the centromeric region of its long arm and a band on the telomeric region of the short arm. No. 12 was characterized by having no definite band except for the centromere.

The variation in bands on some chromosomes of the embryos from different localities was observed, which was shown as dotted lines in the diagram in Fig. 2.

Discussion. A number of investigators have tried to get C- and other bands in various amphibian cells, but it has been difficult to obtain many bands except in Xenopus, Triturus and Rana. Schmid (1978) tried the Q-, G- and R-bandings in the anuran chromosomes and concluded that there were no bands in the euchromatic regions of the metaphase chromosomes. He attributed the results to their strong spiralizations. In cells of Hynobius tokyoensis Tago we were able to obtain some identical banding patterns for the majority of the chromosomes. The findings on the banding patterns may be summarized as follows: (1) We were able to obtain these bands widely in the genus Hynobius, but hardly obtained distinct bands in the chromosomes of the other genus like Onychodactylus japonicus. (2) Even without the treatment of lactic acid, the bands could be obtained just the same to our C-banding treatment. (3) At least, one band was observed on the centromere of each chromosome, even if the chromosomes failed to show many bands with the C-banding treatment. According to the preliminary results in our laboratory, the following two findings should be considered. (4) Staining of distamycin-DAPI for the constitutive heterochromatin showed no specific fluorescent band in chromosomes of this species under the condition of the treatment for human chromosomes. (5) The R-banded chromosomes pretreated with BrdU tended to show the reverse banding pattern of the bands we obtained in this species.

The variation in bands observed on some chromosomes of the
embryos from different localities is a subject for a future study. Whether the variation is attributable to the localization of the animal or to the methods of the treatment would be solved.

**Summary.** The banding patterns of the chromosomes of *Hynobius tokyoensis* Tago are obtained by the treatment of Sumner's BSG method with some modifications. These patterns enable the identification of 17 out of 28 pairs of chromosomes in this species. Morphologically similar chromosome pairs such as nos. 4 and 5, nos. 6 and 7, and nos. 10, 11 and 12 could be easily distinguished from one another.

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**References**