An idiogram on pachytene bivalents with high resolution multiple bands of zebrafish (*Danio rerio*)

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Abstract Well spread pachytene bivalents with high-resolution multiple bands of zebrafish were obtained after the testes were treated with alkaline hypotonic solution and high chloroform fixative solution. This might be the pattern with the largest number of multiple bands obtained from fish chromosomes so far published. Both the number and character of the bands in each bivalent were stable. According to the principles of ISCN (1978) and ISCN (1981), an idiogram of 599 bands was set up, and the detailed description of the landmark system and the band positions were given.

Keywords: zebrafish, bivalents, multiple bands, idiogram.

The longitudinal fine bands of chromosomes demonstrated by the multiple banding techniques, especially, the high resolution banding techniques, can be used to identify individual chromosomes, precisely analyze minor variations of the chromosome structure, and map genes by *in situ* hybridization[1]. Although great progress has been made in the studies on fine chromosome banding in human and other mammals, researches on fish species still lag far behind[2]. Since the middle of the 1970, many attempts have been made to apply these techniques, such as G-banding, R-banding, Q-banding, replication banding and restriction endonuclease banding, to band fish somatic chromosomes[2—6], but only a few were successful. Moreover, neither the resolution nor the reproducibility of the banding patterns had reached a satisfactory extent. So far, only a few idiograms of the banded karyotypes on fish, such as rice field eels[3], Silver carp[6] and Grass carp[7], have been reported.

Zebrafish is popularly used as a model fish species in the studies on vertebrate genetics and development biology[8], and great progress has been made[9,10]. But it is still a trouble to identify individual cytogenetically distinct chromosomes. Former researches demonstrated that the zebrafish *Danio rerio* had 25 pairs of small chromosomes and had no heteromorphic sex chromosomes. Although many recent investigations with a variety of banding techniques, such as C-banding, Ag-NOR banding, G-banding, replication banding and Q-banding, have been successfully applied to zebrafish chromosomes, they have not yet provided the resolution required to identify each chromosome[11—13]. The main obstacles in obtaining excellent multiple banding patterns of zebrafish might be attributed to: (1) all the banding methods used were originally developed to band the

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mitotic chromosome of mammals, and they were not universally suitable to fish mitotic chromosomes, even with some modifications; (2) most fish species have a large number of chromosomes with higher super-coiled degree and smaller size as compared with those of mammals. There will be many favorable factors if the fish meiotic bivalents are used for banding. Firstly, the bivalents can be treated directly with various banding methods without any additional chemical supplements for extending their length. Secondly, the number of chromosomes decreases to half because of homologous pairing in the pachytene. Thirdly, the pachytene passes last quite a long time in fish meiosis cycles, and they tend to be synchronous naturally. Moreover, it has been demonstrated in human and hamster that the chromomere map on bivalent basically corresponds to the high-resolution G-banding patterns of mitosis\textsuperscript{[14,15]}. By this idea, we have successfully developed the high-resolution G-banding patterns of rice field eels\textsuperscript{[16]}. Here we report the first idiogram on the pachytene bivalents with high resolution multiple bands of zebrafish and the detailed descriptions of main characteristics of each individual bivalent chromosome.

1 Materials and methods

1.1 Materials

The testes from twenty adult male zebrafish were used to prepare the bivalents.

1.2 Methods

1.2.1 Preparation of zebrafish bivalents. The zebrafish testes were dissected out alive, subsequently followed by rinsing in 0.85% NaCl. Then, they were treated with hypotonic solution (0.075 mol/L KCl, pH8.0) for 1.5—2 h at room temperature (the hypotonic solution was replaced every 30 min). After the hypotonic treatment, the testes were fixed for 20 min in high chloroform fixative solution (chloroform : methanol : acetic acid, 3 : 6 : 1), and twice refixed in Carnory’s fixative solution (methanol : acetic acid, 3 : 1). The testes were then cut into pieces for preparing the cell suspension which was used to prepare the chromosomal spreads with the conventional air-dry method. The spreads were stained with 10% Giemsa (pH 6.8) for 10 min.

1.2.2 Fluorescent \textit{in situ} hybridization to the centromeres of zebrafish chromosomes. Fluorescent \textit{in situ} hybridization was performed as Amores described in detail\textsuperscript{[11]}. The bivalents preparations were unstained with Carnory’s fixative after being photographed and were refixed with 1% formaldehyde before hybridization. The satellite I DNA derived from genome DNA of zebrafish\textsuperscript{[17]} and labeled with biotin-11-dUTP by random primers was used as probes. 30 μL of hybridization mixture (50% formamide/2×SSC/10% dextran sulfate/50 mmol/L sodium phosphate buffer (pH7.0)/3 μg yeast tRNA/200 ng labeled probes) was denatured for 10 min in boiling water and chilled on ice for 5 min, and then was dropped on the heated-denatured chromosome preparations and covered with a 24×50 mm\textsuperscript{2} coverslip. After hybridization at 37°C in a moist chamber for 20 h and washing of the slides, labeled probes were detected with signal amplification by FITC-avidin