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O-5 Differentially Expressed Genes in Sex Determination of Zebrafish

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INTRODUCTION

The recent interest in the cultivation of the fish species has created a demand for applied studies on methods to improve growth rates as a temporary strategy until domestication and selective breeding originate fast growing strains. One way to improve growth rates currently under consideration is the farming of mono-sex fish. Although a definite proof for the growth rate superiority of mono-sex culture of either sex over mixed culture has yet to be provided, the availability of mono-sex fish seed should allow studies addressing this question as well as provide for the introduction of this species in open water with the reduced danger of uncontrolled reproduction.

In reptiles, incubation temperature is known to control developing sexes(Chanier,1966, ; Bull et al, 1982). Similar results have been obtained in studies on teleost(Harrington,1967 ;Cononer & Kynard,1981). It has been known that the sensitive period for the temperature effect occurs just before metamorphosis. In the zebrafish, all individuals begin to differentiate first as females. Prior to maturation, some portion of them begin to develop testicular tissue. At maturation the sex ratio in the species is 1:1(Takahashi & Shimizu, 1983). However, very few studies have evaluated the role of external factors on sex determination in gonochoristic fish species. Medaka and the guppy exhibit male heterogamety, yet in both species some XX individuals differentiate as male, apparently in response to temperature extremes. This study was planed to find out if and what the temperature has an effect on the sex-ratio in zebrafish. And what genes to be involved in the sex determining process.

MATERIALS & METHODS

The clonal line of zebrafish(C32) which had been obtained from Oregon University and bred in the laboratory, were used as material in the present study. Embryos were produced by artificial insemination and were randomly divided into five temperature groups(22,25,28,32 and 34℃). Each temperature group was randomly subdivided into four incubation periods(early embryonic stage, past embryonic stage, fertilization to sex differentiation stage and fertilization to adult stage).

Total RNA was isolated from zebrafish fry of the groups by Trizol RNA isolation kit(Gibco BRL) as described by the manufacturer. Differential RNA display was performed using a modified protocol of Liang and Paradee(1992). First strand cDNA was synthesized from total RNA. Two different anchor primer were separately used to synthesize cDNA. The sequence of these primers were: Anchor 1 5'-CCGAATTCTTTTTTTTTTTCG-3' and Anchor2 5'-CCGAATTCTTTTTTTTTTTCA-3'. Two different 5' primers were separately used in combination with the two anchor primers. The sequence of the 5'-primers were: 5'-primer1 5'-GCACGGATCCGGTAGATAG-3' and 5'-primer 2 5'-GCACGGATCCTCCTGATCCATG-3'. PCR reactions were subjected to 30cycles of 1min at 94℃, 1min at 55℃, and 1min at 72℃. PCR reactions were run in 6% denaturing polyacrylamide gel. Following electrophoresis, the gel was autoradiographed and bands were excised. Individual eluted bands were amplified by PCR and checked on agarose gel. The remaining PCR reaction was then cloned into the pGEM vector as described by the manufacturer, and sequenced.

RESULTS & DISCUSSION

The mean survival rate at the time of sexing was not differ significantly between the control batches reared in temperature of 28.5℃ and the batches subjected to temperature treatment. No significant difference was observed in the sex ratio among batches of the same treatment group. Seventeen treatment groups of fry, each divided into several batches, were reared five temperature treatments. Compared to controls, four of these batches(temperatures were treated fertilization to adult stage) showed different sex ratios. These

sex ratio was not caused by differential mortalities related to high or low temperature. In the control temperature batch(28.5℃), average sex ratio was female 7, male 3. The batch exposed to low temperature(22℃ and 25℃) showed increased female ratio, female 3.8 : male 6.2 and 5 : 5 each. Two batches exposed to 32℃ and 34℃, sex ratios were significantly biased toward the males, female 1 : male 9 and 0.2 : 8.2 each, compared to the percentages observed in the control batches exposed to 28.5℃. This results suggest that the sex ratios of high or low temperature treated zebrafish fry are directly influenced by the rearing temperature.

The differential RNA display has been used to identify genes specifically expressed during sex differentiation stages and to find out their relationship. RNA from zebrafish which were reared at temperature 34℃ and 22℃ was compared using the differential RNA display. Putative differential bands were excised from the gel, reamplified and cloned. Using combinations of four oligonucleotides, a total of 19 bands were selected and sequenced from differential display. This cloned genes are under analysis. The results demonstrates that early rearing temperature is responsible for the sex determination for the sex determination process under the condition used.

SUMMARY

The effects of rearing temperature on the sex determination of zebrafish were examined to address the questions, " Is it possible to control phenotypic sex artificially; and what genes may be involved in that process?". At higher temperature(32-34℃ vs 28.5℃), a dramatic shift of the sex ratio(female 1 : male 9) was found. With DD-PCR cloning, several candidate genes were cloned and analyzed. Further analysis should help to understand sex-determining mechanisms involved in the artificial induction.

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