Molecular biological studies on temperature acclimation of fish cultured cells

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INTRODUCTION

While fish cell cultures have been used for various applications, temperature acclimation of cultured fish cells is one of the major subjects. Bols *et al.*¹⁾ claimed that fish cells could survive and proliferate in the temperature range where fish grow. For example, RBCF-1, which was derived from goldfish caudal fin by Shima *et al.*,²⁾ could grow in the temperature range of 20 to 37 C. Tsugawa and Lagerspetz³⁾ reported that the membrane fluidity of another goldfish cell line, CAF, cultured at 34 C was lower than that cultured at 14 C. These results indicate that goldfish cultured cells can easily adapt to different environmental temperatures, however, the molecular mechanisms involved in temperature acclimation of fish cells are still not clear.

In this report, we cultured cells derived from goldfish caudal fin at 20, 25, 30, and 35 C, and their growth rates were examined. Furthermore, mRNAs differentially expressed in a temperature-dependent manner were cloned and their sequences determined.

MATERIALS AND METHODS

The caudal fin of goldfish was sterilized in 0.4% NaClO containing 70% ethanol. The tissue pieces were washed with the Hanks' balanced salt solution, and then added with 10 mL of L-15 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS), streptomycin (100 µg / mL), and penicillin (100 U / mL). The tissue pieces were placed into 100-mm² dish and incubated at 35 C for 10 days. Subsequently, cell monolayers explanted from the

tissue pieces were treated with trypsin and resulting cell suspension was used for cell cultures at 20, 25, 30, and 35 C in the medium containing 5% FBS and 5% carp serum (CS).

mRNA from cells cultured at 20 and 35 C was purified by Oligotex-dT30 super (Takara), followed by cDNA synthesis with cDNA synthesis kit (Takara) which was performed according to the manufacturer's protocols. cDNA-representational difference analysis (cDNA-RDA) was performed according to Lisitsyn *et al.*⁴⁾ Temperature-specific cDNA fragments were subcloned and their nucleotide sequences determined. Furthermore, temperature-dependent changes of mRNA levels of the genes concerned were confirmed by northern blot analysis.

RESULTS AND DISCUSSION

Cells derived from goldfish caudal fin showed a fibroblast-like morphology and grew in the culture medium containing 10% FBS. The effects of serum species on primary cell cultures were investigated in the temperature range of 20 to 35 C. It has been reported that CS was effective on the proliferation of cultured goldfish cells in early passage⁵⁾. While the cells in the medium containing 10% FBS showed poor growth at 20-30 C, those in the medium supplemented with 10% CS or 5% FBS plus 5% CS grew well in the temperature range of 20 to 35 C. The medium containing 5% FBS plus 5% CS enhanced cell growth more effectively than that containing 10% CS only. Thus, the former medium was used for subsequent cell culture, demonstrating that cells at 20, 25, 30 and 35 C grew with population

doubling times of 36, 35, 20 and 18 h, respectively.

The population doubling time was markedly different between cells culture in the temperature range of 20 to 25 °C and those in the range of 30 to 35 °C as described above. As goldfish normally live below 30 °C, a higher growth rate at and over 30 °C suggests that goldfish cultured cells acquired certain survival mechanisms at such high temperatures. To elucidate such mechanisms of growth over 30 °C, cDNA-RDA was performed for cells cultured at 20 and 35 °C. Twenty cDNA fragments were differentially expressed in a culture temperature-dependent manner. These included the genes encoding calmodulin and ATP translocase which were specific to cells cultured at 20 °C, although their expression levels were not high. On the other hand, the transcripts encoding type I collagen α chain (col α (I)) were most significantly increased in cells at 35 °C compared to those at 20 °C. Type I collagen is the most abundant extracellular protein, and endows certain cells with tolerance to heat shock⁶⁾. Therefore, it can be speculated that type I collagen in goldfish cells participates in cell survival under high temperature stress conditions.

The time-dependent change of the col α (I) mRNA level was further investigated in cells cultured at 20 and 35 °C. The levels gradually increased during cell cultures at both 20 and 35 °C, and the levels at 35 °C were usually higher than those at 20 °C at any sampling time, resulting in a higher rate of increase of the collagen mRNA levels at 35 °C. Both the accumulation rate of the collagen mRNA and growth rate were increased when the temperature was shifted from 20 to 35 °C. The inverse tendency was observed when the culture temperature was shifted from 35 to 20 °C. It has been reported that type I collagen gene expression is enhanced by growth factors and hormones⁷⁾, and as such there might be temperature-specific growth factors that regulate cell

growth and collagen gene expression in goldfish.

In conclusion, cultured cells from goldfish could grow in a wide temperature range (20-35 °C). High cell growth rate and rapid accumulation of col α (I) mRNA were observed in cells cultured at and over 30 °C. As such high temperatures are considered to be stressful for goldfish, differences in stress responses against high temperatures between cultured cells and intact body are needed to be investigated in more detail.

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