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Identification of differential transcript profiles between mutual crossbred embryos of zebrafish (*Danio rerio*) and Chinese rare minnow (*Gobiocypris rarus*) by cDNA-AFLP

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Abstract

The crosstalk between naive nucleus and maternal factors deposited in egg cytoplasm before zygotic genome activation is crucial for early development. In this study, we utilized two laboratory fishes, zebrafish (*Danio rerio*) and Chinese rare minnow (*Gobiocypris rarus*), to obtain mutual crossbred embryos and examine the interaction between nucleus and egg cytoplasm from different species. Although these two types of crossbred embryos originated from common nuclei, various developmental capacities were gained due to different origins of the egg cytoplasm. Using cDNA amplified fragment length polymorphism (cDNA-AFLP), we compared transcript profiles between the mutual crossbred embryos at two developmental stages (50%- and 90%-epiboly). Three thousand cDNA fragments were generated in four cDNA pools with 64 primer combinations. All differentially displayed transcript-derived fragments (TDFs) were screened by dot blot hybridization, and the selected sequences were further analyzed by semi-quantitative RT-PCR and quantitative real-time RT-PCR. Compared with ZR embryos, 12 genes were up-regulated and 12 were down-regulated in RZ embryos. The gene fragments were sequenced and subjected to BLASTN analysis. The sequences encoded various proteins which functioned at various levels of proliferation, growth, and development. One gene (ZR6), dramatically down-regulated in RZ embryos, was chosen for loss-of-function study; the knockdown of ZR6 gave rise to the phenotype resembling that of RZ embryos.

Keywords: Zebrafish; Chinese rare minnow; Crossbred embryo; cDNA-AFLP; Differential expression

1. Introduction

In previous studies of animal cloning, the majority of intra-species nuclear transfers (NTs) yielded cloned individuals identical to their nuclear donor species either on the basis of phenotype or genotype [1–5]. However, this was not the case for cross-species NT, wherein enucleated eggs cytoplasm had an obvious impact on the mitochondrial genetic materials and the development of NT embryos [6,7].

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Our previous study revealed that the somitogenesis and vertebral number of the cross-genus cloned fish resembled those of the cytoplasmic recipient species, goldfish, instead of the donor nuclear species, common carp [6]. Therefore, the crosstalk between the donor nucleus and the recipient egg cytoplasm from different species could modify the early development of NT embryo, possibly by affecting gene expression of the transplanted nucleus. Studies on inter-species NT between bovine and pig likewise demonstrated that the embryonic development was driven by the host oocyte, up to the stage when zygotic genome activation should occur [8]. Therefore, it is necessary to develop an appropriate research system to study the impact on the transplanted nucleus induced by egg cytoplasm from another species. As described in our previous study, we utilized cross-species NT to address this question [9]. However, there were two scientific issues, i.e., nuclear reprogramming of the donor nucleus and interaction between the nucleus and cytoplasm from different species. This presented a difficulty in distinctly clarifying genes that were solely involved in the nucleo–cytoplasmic interaction, prompting us to seek a novel system.

Unlike higher vertebrates, fishes could be easily used to produce crossbred embryos between two different species. In the present study, we utilized two laboratory fish species, zebrafish and Chinese rare minnow, to obtain two types of mutual crossbred embryos, zebrafish ♀ × Chinese rare minnow ♂ (ZR) and Chinese rare minnow ♀ × zebrafish ♂ (RZ). As one of the model animals, zebrafish (*Danio rerio*) has an important role in the basic research of genetics and developmental biology [10]. The Chinese rare minnow (*Gobiocypris rarus*), meanwhile, is a small cyprinid fish which has proved to be an ideal species for laboratory study [9]. Given that the two types of mutual crossbred embryos have the same composition of nucleus but different egg cytoplasm, they could provide us with novel materials to study cross-species nucleo–cytoplasmic interaction. More importantly, when compared with cross-species NT, since there are no issues related to nuclear reprogramming in this event, mutual crossbred embryos are more appropriate for the study of nucleo–cytoplasmic interaction.

The differential expression analysis approach of cDNA amplified fragment length polymorphism (cDNA-AFLP) is based on the selective PCR-amplification of adapter-ligated restriction fragments derived from cDNA [11]. It is proven to be a powerful transcript profiling approach for genome-wide analysis without the need for prior sequence knowledge [12]. In this study, we applied the cDNA-AFLP approach to identify differential transcript profiles between ZR and RZ embryos.

2. Materials and methods

2.1. Experimental fish and embryos

Zebrafish and Chinese rare minnow were raised in our laboratory. Crossbred embryos ZR and RZ were obtained by artificial fertilization and cultured to a specific stage for RNA and DNA isolation. Embryos of the zebrafish, the Chinese rare minnow, and the crossbred varieties were incubated at 28, 25, and 26 °C, respectively. To compare the developmental timing of these embryos, they were incubated at 26 °C and staged according to the literature descriptions [13].

2.2. Extraction of total RNA and DNA

Total RNA was extracted using the SV Total RNA Isolation System (Promega, Madison, WI, USA) from the crossbred embryos at 50%- and 90%-epiboly stages, and zebrafish or Chinese rare minnow embryos at the 50%-epiboly stage. The integrity and concentration of the total RNA were verified by electrophoresis and quantified with a spectrophotometer.

For DNA isolation, larval fish were lysed with 300 μL extraction buffer (10 mmol/L Tris–Cl, pH 8.0, 0.1 mol/L EDTA, pH 8.0, 0.5% SDS, 10 mg/mL Proteinase K), homogenized, and incubated at 55 °C for 3 h. Total DNA was extracted by phenol/chloroform, purified by ethanol precipitation, and dissolved in TE buffer (10 mM Tris–Cl, pH 7.5, 1 mM EDTA). The DNA concentration was estimated through agarose gel electrophoresis and stored at −20 °C.

2.3. Molecular confirmation of the crossbred embryos

Before cDNA-AFLP was performed, total DNA from the crossbred embryos was PCR-amplified with a species-specific sequence characterized amplified region (SCAR) primers of zebrafish and Chinese rare minnow [14] (Table 1). The SCAR PCR with appropriate primers was expected to yield a 500 bp fragment in the Chinese rare minnow and a 248 bp band in the zebrafish. The PCR reactions included 100 ng DNA, 0.2 mM dNTP, 1× LA buffer, 0.5 U LA Taq (Takara, Dalian, China), and 10 mM of each primer. The PCR parameters were as follows: a pre-denaturation of