NUCLEAR TRANSPLANTATION IN DIFFERENT FAMILIES OF TELEOST : COMBINATION OF THE NUCLEUS OF ZEBRAFISH (BRACHYDANIO RERIO) AND THE CYTOPLASM OF LOACH (PARAMISGURNUS DABRYANUS)

Y. SHAOYI*
M. ZHONGRUNG*
L. SHUKHONG*
H. GUOPING*
C. HUIPING*
J. GUANQUIN*
L. LIDONG*
X. SHILING*
Y. LEINING*
X. DEQUAN*

SUMMARY : In this paper, we present the results obtained in the experiments of nuclear transplantation in teleost of different families. The nuclei of blastula cells of zebrafish (Brachydanio rerio, family Cyprinidae, order Cypriniformes, 2N=50) were transplanted into the enucleated eggs of loach (Paramisgruns dabryanus, family Cobitidae, order Cypriniformes, 2N=48). From 4812 nucleocytoplasmic hybrid (NCH) eggs eight NCH larval fish were obtained (0.17%). One of which developed up to 151 hours. The developmental potential of this kind of NCH eggs was improved as compared to the NCH eggs obtained in other inter-family combinations reported by us previously, i.e. the combination of the nucleus of goldfish (Carassius autratus, family Cyprinidae, order Cypriniformes, 2N=100) and the cytoplasm of loach (Paramisgurnus dabryanus, family Cobitidae, order Cypriniformes, 2N=48) and their reciprocal combination, the combination of the nucleus of loach and the cytoplasm of goldfish. The results obtained in this experiment indicated that the similarity of the chromosome number between the nucleus-donor and cytoplasm-recipient fishes will be helpful for improving the natural developmental incompatibility between the nucleus and the cyptoplasm in an NCH egg, even though they originate from two taxonomically distantly related species of fishes.

Key Words : Nuclear transplantation, different orders, teleost.

INTRODUCTION

Our previous papers reported the results obtained in the experiments of nuclear transplantation in teleosts from different families : (1) The nuclei of goldfish (Carassius auratus, family Cyprinidae, order Cypriniformes, 2N=100) were transplanted into the enucleated eggs of loach (paramisgurnus dabryanus, family Cobitidae, order Cypriniformes, 2N=48), and (2) the nuclei of loach were transplanted into the enucleated eggs of goldfish. In both cases, only few early nucleo-cytoplasmic hybrid (NCH) larval fish were obtained. For example, one latest NCH larval fish obtained in the first case only developed up to

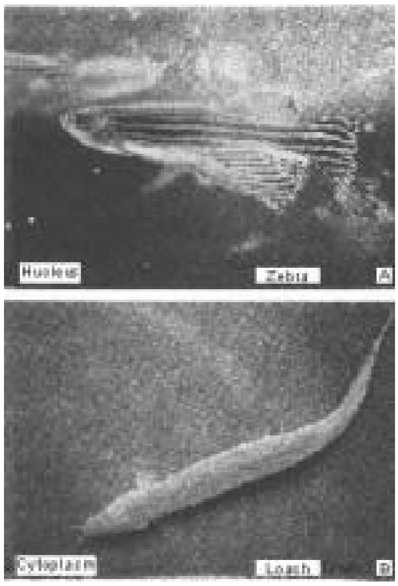
*From Department of Physiology, Faculty of Medicine, University of Çukurova, 01330, Adana/TURKEY.

43.5 hours and the other one obtained in the second case developed up to 45 hours. The results obtained in those two combinations indicated that the developmental potential of their NCH eggs were almost restricted at the same early larval fish stage (3).

On the other hand, when the nuclei and the cytoplasm in the NCH eggs were obtained from two more distantly related species (different orders) some different results were obtained. For example, when the nuclei of tilapia (Oreochromis nilotica, order Peciformes, 2N=44) were transplanted into the enucleated eggs of goldfish (Carassius auratus, order Cypriniformes, 2N=100) only NCH blastulae or few early NCH gastrulae were obtained (3) whereas when the nuclei of tilapia (Oreochromis nilotica, order Peciformes, 2N=44) were transplanted into the enucleated eggs of loach (Paramisgurnus dabryanus, order Cypriniformes, 2N=48), one larval fish developed up to 6th day and the other one developed up to 12th day. Both larval fish seemed to have their normal developmental features before they died (4). These results indicated that even though in some inter-order combinations, the developmental potential of the NCH eggs could become better than those observed either in two inter-family and one inter-order combinations previously reported (3).

The interpretation of those phenomena may be as follows: According to the results previously reported in a series of our nuclear transplantation experiments in teleost of different varieties, genera, subfamilies, families and orders, we have observed that the incompatibilities between the cell nucleus and the egg cytoplasm from two distantly related species of fish in an NCH egg is the main factor which should determine the developmental potential of the NCH eggs (2). However, there are also other factors which may influence the developmental fate of the NCH eggs as well. For example, the difference in chromosome number between the nucleus-donor and the cytoplasm-recipient fish species may provide one of the other essential factors influencing the extent of development of NCH eggs irrespective of how far the two kinds of fishes are taxonomically classified (2,3). In addition, the feeding behavior of the NCH larval fish may also differ from those of their normal nucleus-donor or cytoplasm-recipient fishes. So, it might be another important factor which may also prevent the NCH larval fish from growing into adults (5).

Figure 1: Pictures of zebrafish (Brachydanio rerio, family Cyprinidae, order Cypriniformes) (A) and loach (Paramisgurnus dabryanus, family Cobitidae, order Cypriniformes) (B).



In order to obtain more information for confirming the above mentioned hypothesis, we looked for the proper combinations of candidate fishes of different families or orders which not only have similar chromosome numbers but also have similar developmental feeding behaviors. The zebrafish and loach seemed to be a pair of good candidate fishes for this purpose.

The objective of this paper is to present the results obtained from the combination of the nucleus of zebrafish (Brachydanio rerio, family Cyprinidae, order Cypriniformes, 2n=50) and the egg cytoplasm of loach (Paramisgurnus dabryanus, family Cobitidae, order Cypriniformes, 2N=48) by using nuclear transplantation method. In this combination, both kinds of fish belong to the same order but to different families. They have similar number of chromosomes, pattern of embryo genesis and feeding behavior of their larval fish. The pictures of both fish are shown in Figure 1.

Table 1: The survial rate of development stage of NCH eggs which were obtained from the combination of the nucleus of zebrafish and the cytoplasm of loach.

No. of transplanted eggs	No. of blastula stage	No. of gastrula stage	No of larval fish	No. of adult fish
4812	3225	161	8	0
(100%)	(67%)	3.34%)	(0.17%)	(0%)

MATERIAL AND METHODS

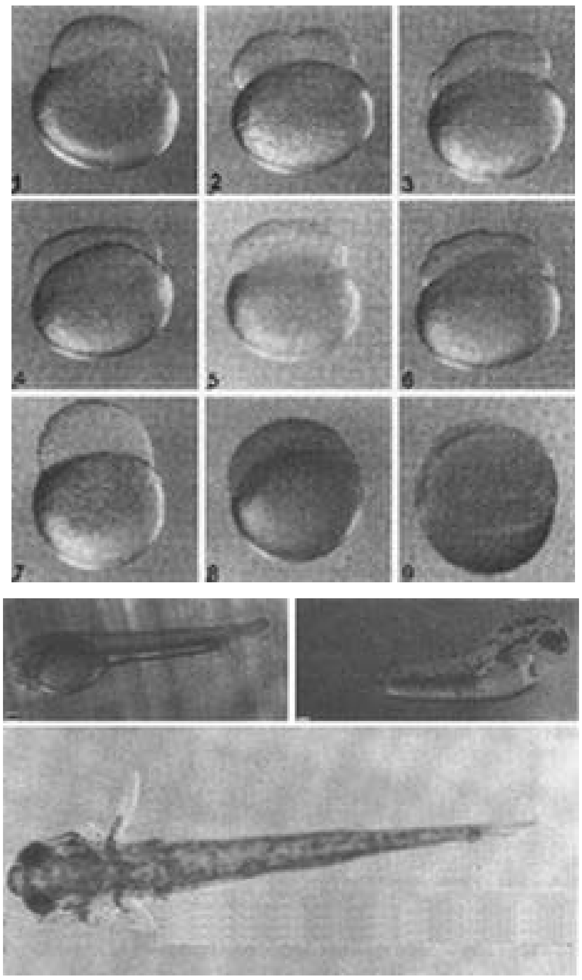
Fish stocks and technique for nuclear transplantation and chromosome examination

The zebrafish and loach were provided by the aquaculture facilities of the Institute of Developmental Biology, The Chinese Academy of Sciences, Beijing, China. The method for nuclear transplantation in teleost was reported in a previous paper (2). The method for chromosome examination was according to Yamazaki's method (1).

RESULTS

In this experiment, among total of 4812 nucleus-transplanted eggs 3225 developed into blastula stage (67%), 161 developed into gastrula stage (3.34%) and 8 developed into larval fish stage (0.17). One of them developed up to 151 hours and then died. No NCH adult fish was obtained. The results obtained in this

Figure 2: The early development of NCH eggs obtained from the combination of the nucleus from zebrafish and the cytoplasm from loach. 1. 1-cell, 2. 2-cell, 3. 4-cell, 4. 8-cell, 5.16-cell, 6. 32-cell, 7. early blastula, 8. late blastula, 9. middle gastrula, 10. 30 h and 45 min. larval fish, 11. 70.5 h larval fish, 12. 151 h larval fish.



combination were summarized in Table 1.

The pictures of the early normal development of the NCH eggs obtained in this combination are seen in Figure 2, 1-12. The chromosome examination of 14 NCH blastulae showed that their chromosome number is 50, i.e. the type of zebrafish (Figures 3, 2b).

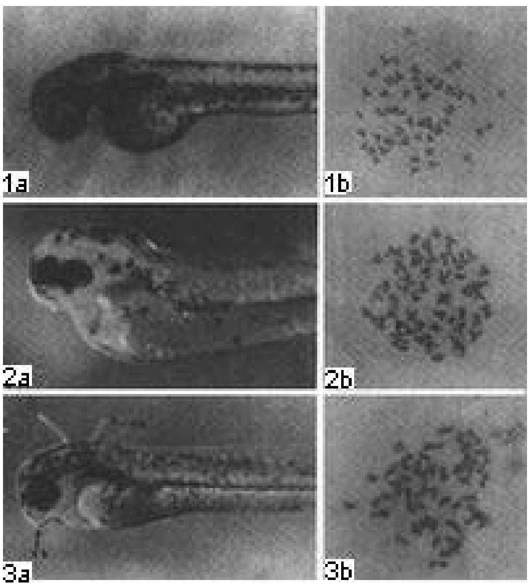
Morphological examination of the NCH larval fish as well as the larval fish of zebrafish and loach showed that no embryonic external gills or oral barb rudiments were detected in the NCH larval fish (including the one developed up to 151 hours), whereas five pairs of embryonic external gills and three pairs of oral barb rudiments were clearly detectable in the loach larval fish at similar developmental stage. Neither embryonic external gills nor oral barb rudiments have appeared in zebrafish larval fish during its normal development.

Figure 3 shows the comparison of morphological characteristics and the metaphase pictures among the larval fish of zebrafish (1a, b), the NCH larval fish obtained from the combination of the nucleus of zebrafish and the cytoplasm from the combination of the nucleus of zebrafish and the cytoplasm from loach (2a, b) and the larval fish does not have any oral barb rudiment or embryonic external gills, it should be a true NCH larval fish developed from the combination of the nucleus of zebrafish and the cytoplasm of loach. This larval fish has all its internal and external organs. The pigmentation on the skin seemed also normal. It could swim slowly but sometimes it could not maintain its normal balance. Its cardiac rate and rhythm were normal. Its blood cells were red in color but its blood circulation was blocked before it died after 151 hours of survival. The mouth was formed without any normal function for catching food.

DISCUSSION

The results obtained in this combination (the nucleus from zebrafish and the cytoplasm of loach, an

Figure 3: The comparison of morphological characteristics among the larval fish of zebrafish, the NCH larval fish obtained from the combination of the nucleus from zebrafish and the cytoplasm from loach, and loach. 1a. A 55 h larval fish of zebrafish without oral barb rudiments and embryonic external gills.1b.A metaphase of zebrafish blastula (2N=50). 2a. A 72 h NCH larval fish without oral barb rudiments and embryonic external gills. 2b. A metaphase of NCH blastula (2N=50). 3a. A 49 h larval fish of loach with oral rudiments (As shown by arrow marked with o.b.) and embryonic external gills (As shown by arrow marked with external gills on right side of the head were shown and the other three of them were covered by the head of the fish. The five embryonic external gills on the left side of the head were out of the focus in this picture). 3b. A metaphase of loach blastula (2N=48).



inter-family combination) were compared with those obtained from following combinations which were previously reported. They are two inter-family combinations : (1) The nucleus of goldfish, Carrassius autratus, family Cyprinidae, order Cypriniformes, 2N=100 combined with the cytoplasm of loach, Paramisgurnus dabryanus, family Cobitidae, 2N=48, and (2) the nucleus of loach combined with the cytoplasm of goldfish (3). Two inter-order combinations (3) the nucleus of tilapia, Oreochromis nilotica, order Peciformes, (2N=44) combined with the cytoplasm of loach, Paramisgurnus dabryanus, order Cypriniformes, (2N=50) and cytoplasm recipient (loach, 2N=48) have similar number of chromosomes, the developmental potential of their NCH eggs became much better than those NCH eggs obtained from aforesaid other two inter-family combinations (combinations 1 and 2) and one inter-order combination (combination 3). In those combinations the nucleus-donor and cytoplasm-recipient fishes had different chromosome numbers (3). However, the developmental potential of the NCH eggs obtained in this combination are also similar to that of the NCH eggs obtained from another inter-order combination (combination 4) in which the chromosome numbers of the nucleus-donor fish, tilapia, (2N=44) and that of the cytoplasm-recipient fish, loach, (2N=48) is similar (4).

These results are also in line with those already observed in the NCH eggs obtained from the combinations of the nucleus and the cytoplasm of different genera of fish (the nucleus of common carp, Cyprinus carpio, genus Cyprinus Linnaeus, 2N=100) and those of different subfamilies (the nucleus of grass carp, Carassius auratus, genus Carassius Jarock, 2N=48) combined with the cytoplasm of blunt-snout bream, Megalobrama amblycephala, subfamily Abramidinae, 2N=48). In those two combinations, the number of chromosomes of nucleus-donor and cytoplasm-recipient fishes were the same and NCH adult fish were obtained.

Therefore, the results obtained in this inter-family combination also supported the speculation that the similarity of the chromosome number between the nucleus-donor and the cytoplasm-recipient fishes is one of essential factors which may improve the developmental potential of NCH eggs.

As to the factor of feeding behavior, there is no obvious difference found between the larval fish of zebrafish and loach. For example, both kinds of larval fish hatched between 48 to 72 hours after fertilization at 26 °C and similar amounts of yolk remained in their abdominal cavities as the nutritional sources for their further development. The remaining yolks were utilized by both larval fish almost at similar stages when they developed into 5th or 6th days after their mount were well developed for catching food. Morphologically, the NCH larval fish developed up to 151 hours in this study looked similar to those of larval fish developed either from normal zebrafish or loach eggs. This 151 hours NCH larval fish was expected to have the same capability to catch food for supporting its further development as the normal larval fish of zebrafish and loach. But the mouth of this NCH larval fish did not have normal function. Therefore, there is no good evidence, at least in this combination, to support that the poor feeding behavior of this 151 hours NCH larval fish is another important factor which may prevent its further growth as it was previously reported (5).

Instead, some unknown factors related to the incompatibility between the nucleus and cytoplasm (which were obtained from distantly related fish species within the NCH eggs seems still performing their strong rules for preventing the NCH larval fish from further normal development and the nature of those factors remained to be investigated in the future.

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Correspondence:
Yan Shaoyi
Çukurova Üniversitesi
Tip Fakültesi
Psikoloji Bölümü
01330, Adana/TÜRKIYE