Reproduction and Breeding 1 (2021) 81–88

Contents lists available at ScienceDirect

Reproduction and Breeding

journal homepage: www.keaipublishing.com/en/journals/reproduction-and-breeding

Characteristics of hatching enzymes and egg envelope in cross progenies from crucian carp (*Carassius auratus var.*) and zebrafish (*Barchydanio rerio var.*)

Wen Fu [a,b], Jinping Zhang [a,b], Wenting Xu [a,b], Sheng He [a,b], Mindi Long [a,b], Qingqing Liao [a,b], Jinhui Liu [a,b], Liangyue Peng [a,b], Wenbin Liu [a,b], Yamei Xiao [a,b,*]

[a] State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha, Hunan, 410081, PR China
[b] College of Life Sciences, Hunan Normal University, Changsha, Hunan, 410081, PR China

ARTICLE INFO

Keywords:
Fish
Distant hybridization
Hatching
Envelope
Enzyme

ABSTRACT

In distant hybridization of fishes, there is a low hatching rate of hybrid progenies. To further understand the causes of low hatchability, in this article, cross-breeding between zebrafish (ZF) and crucian carp (CC) are generated, in which the hatching rate of cross progenies (ZF♀ × CC♂, ZC) is 35.67%, while that of reciprocal cross progenies (CC♀ × ZF♂, CZ) is about zero. The structures and SDS-PAGE protein pattern of egg envelopes in hybrid early embryos are like their maternal fish, however, during the process of embryo development, some differences are detected between the CZ and CC. The hatching enzyme gene 1 (*he1*) nucleotide sequences from ZF and CC were 85.1% identity, while that of 99.6% identity was between the ZF and ZC, and 98.4% from CC and hybrid of CZ. There are differences in predictive protein physicochemical properties of he1 between CZ and CC, but no significant change in *he1* mRNA levels during embryonic development. Our data furtherly verify that the characteristics of egg envelope and hatching enzyme in hybrid progenies are inherited from maternal fish. Moreover, there are obvious differences between CZ and maternal parent in both egg envelope and hatching enzyme during embryonic development, which are considered to be related to its low hatching rate.

1. Introduction

Hybridization, or cross-breeding, is the result of combining the qualities of two organisms of different breeds or species through sexual reproduction. According to the parental genetic relationship, hybridization can be categorized into two types, close hybridization and distant hybridization. Breeding practices confirmed that the distant hybridization, a cross between parents that differ by species or higher classifications, has a greater potential for breeding new groups and even new species [1–7]. However, there is a low hatching rate in distant hybrid progenies [2].

As an oviparous (egg-laying) animal, fish embryo develops within the egg envelope. Kendall et al. [8] believed that the period from spawning to hatching of fish should be an egg stage instead of using an embryonic stage, due to there are aspects to do with the egg envelope besides embryonic development. Egg envelope, a non-cellular envelope, is the protective adventitia of embryos [9]. In fish, egg envelope has several layers, usually the outer layer is thinner, and the inner layer is thicker [10–12]. After the embryo develops to a certain stage, it must be freed from the envelope to further grow and develop, which is named hatching [13]. If the envelope breaking is not timely, the growth and development of the fish will be seriously disrupted, resulting in the decrease of hatching rate and growth rate.

It is generally considered that egg envelope breaking is involved two mechanisms, i.e., mechanical hatching and enzymatic hatching [14]. The mechanical hatching of fish is mainly through the movement of embryos, such as the flexion and extension of the embryo body [15]. The enzymatic hatching is the process of enzymatic choriolysis by hatching enzyme, secreting from the gland cells [16,17]. But there reported that embryos inhibited by exercise could still hatch [18]. Thus, it is generally believed that enzymatic hatching plays a decisive role in the hatching process of fish [18,19].

Crucian carp (*Carassius auratus*), a common omnivorous freshwater fish in Eurasia, is an important economic aquaculture fish. By hybridization, some new breeds of aquaculture have been obtained, such as gibel carp (*Carassius auratus gibelio*♀ × *Cyprinus carpio*♂) [20], allotriploid

* Corresponding author. State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha, 410081, China.
E-mail address: yameix@hunnu.edu.cn (Y. Xiao).

https://doi.org/10.1016/j.repbre.2021.04.001
Received 12 November 2020; Received in revised form 9 April 2021; Accepted 19 April 2021
Available online 1 May 2021

crucian carp (*Carassius auratus red var*♀ × *allotetraploid Carassius auratus*♂) [21], and hefang crucian carp (*Carassius auratus cuvieri Temminck et Schlegel*♀ × *Carassius auratus red var*♂) [22]. However, the phenomenon of low hatchability was still widespread in distant hybridization [2]. Due to strong reproductive capacity, short sexual maturity cycle, especially large-scale positive gene saturation mutation and screening, zebrafish is one of the important model vertebrates in revealing the molecular mechanism of embryonic and tissue organ development, and yet establishing toxicological and aquatic breeding models to study of environmental and agricultural problems. In this study, we designed a cross-breeding between crucian carp (*Carassius auratus*, 2n = 100, abbreviated as CC) and zebrafish (*Barchydanio rerio* var, 2n = 50, abbreviated as ZF). Considering the critical effects of egg envelope and hatching enzyme on embryo hatching, we investigated the characteristics of egg envelope and hatching enzymes among parents and their hybrid progenies, not only the crosses (Zebrafish♀ × Crucian carp♂), but also reciprocal crosses (Crucian carp♀ × Zebrafish♂). The results on the characteristics of egg envelope and hatching enzyme of distant hybridization offspring may be helpful to distant hybridization breeding.

2. Materials and methods

2.1. Ethics statement

Animal experimenters were licensed after attending a training course on laboratory animals held by the Institute of Experimental Animals, Hunan Province, China. Fish work was performed in strict accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the National Advisory Committee for Laboratory Animal Research in China and was approved by the Animal Care Committee of Hunan Normal University.

2.2. Fish

Zebrafish and crucian carp were maintained at the State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University. The fertilized zebrafish eggs and the cross progenies (zebrafish♀ × crucian carp♂, abbreviated as ZC) were cultured in 28 °C water, however, the fertilized eggs of crucian carp (CC) and the reciprocal cross progenies (crucian carp♀ × zebrafish♂, abbreviated as CZ) were in 24 °C water.

2.3. Preparation of scanning electron microscope samples

Pieces of fresh envelopes were torn from different stage eggs by sharp tweezer. For electron microscopy, the envelope pieces were fixed at 4 °C in 2.5% glutaraldehyde solution for 2 h, then, treated by 1% OsO4 for 1 h. After dehydration through an ethanol series, the specimens were treated by the freeze dryer (Japan, JFD-310), sprayed with gold (JFC-1600, Japan), then were examined by scanning electron microscope (JSM-6360LV, Japan).

2.4. SDS-PAGE analysis of egg envelope proteins

Egg envelopes were collected separately from six different stage embryos, e.g. blastula, gastrula, optical vesicle, muscle differentiation, eye pigmentation, and body pigmentation. They were subsequently digested in SDS-PAGE (dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis) sample loading buffer by boiling in water, then, handled with the ultrasonic cell crusher. The SDS-PAGE, 10% (w/v) separating gel and 4% (w/v) stacking gel, was carried out according to the procedures described by Laemmli [23]. After electrophoresis, the gel was stained with Coomassie Brilliant Blue G −250.

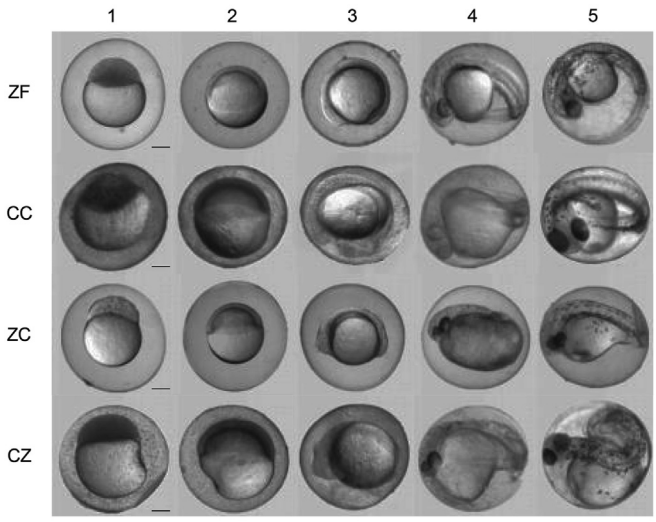

Fig. 1. Hybrid embryos of zebrafish (ZF) and crucian carp (CC). ZC was the hybrids of cross-ZF (♀) × CC (♂), and CZ was the hybrids of reverse cross-CC (♀) × ZF (♂). 1 to 5 were the embryos at the stages of blastula, gastrula, optical vesicle, eye pigmentation, and body pigmentation, respectively. The scale bar of ZF and ZC = 0.2 mm, and scale bar of CC and CZ = 0.3 mm.

2.5. RT-PCR

Total RNAs were extracted from eggs at different embryo stages (e.g. gastrula, optical vesicle, muscle differentiation, eye pigmentation, and body pigmentation) using Trizol Reagent (TaKaRa, Japan) following the manufacturer's protocol. In the reverse transcription-linked polymerase chain reaction (RT-PCR), β-actin primers were used as control to determine expression of the HE (hatching enzyme) gene, and the reaction was performed for 30 cycles. All the primers used in this study were listed in Table S1. Each experiment was repeated more than 3 times.

2.6. Molecular cloning of hatching enzyme CDS (coding sequence)

A reverse transcription linked polymerase chain reaction (RT-PCR) cloning technique was used to clone the hatching enzyme cDNA from crucian carp and hybrids. The RT-PCR was conducted with a kit (Invitrogen, USA) with 2 μg total RNA. The PCR was followed by 30 cycles of 94 °C for 30s, 50 °C for 30s, and 72 °C for 60s. The amplified products were gel-purified and cloned into pMD18-T vector (TaKaRa, Japan), and the obtained pMD18-HE-T plasmid was used for DNA sequencing.

2.7. Plasmid construction

The p-EGFP-N1 plasmid as vector backbone, digested with restriction enzymes Nhe I and Hind III (Thermo, USA) and purified. The pMD-HE-18-T plasmid contains the HE gene CDS, and digested with restriction enzymes Nhe I and Hind III (Thermo, USA), after purification, the CDS fragment of the HE gene was obtained. The p-EGFP-N1 backbone and HE gene CDS fragment were ligated by T4 ligase (TaKaRa, Japan) to create the new plasmid, hereafter referred to as p-HE-EGFP-N1.

2.8. Plasmid microinjection

The fertilized eggs of *carassius auratus* was predigested with 0.25% trypsin solution for 3min, then were in a 1.5% agar plate containing Holtfreter's solutions. The plasmid was diluted with DEPC (diethyl pyrocarbonate) water to 80 ng/μl and placed on ice for further used. The fertilized eggs were injected under the OLYMPUS microinjector (SZX-ILLK200, Japan) before the first cleavage.

Table 1
Fertilization rate and hatching rate of Zebrafish (ZF), Crucian carp (CC), Zebrafish ♀ × Crucian carp ♂ (ZC) and Crucian carp♀ × Zebrafish♂ (CZ).

Group	Fertilization, (%)	Hathing, (%)
ZF	93.40%	97.80%
	91.80%	97.16%
	92.77%	95.55%
CC	84.62%	95.03%
	88.49%	97.75%
	91.56%	95.90%
ZC	92.56%	34.23%
	94.55%%	36.77%
	92.80%	36.00%
CZ	76.82%	0
	80.71%	0
	80.68%	0

3. Results

3.1. Hybrid progenies of crucian carp and zebrafish with low hatchability

Crossings were performed by zebrafish (ZF) and crucian carp (CC) (Fig. 1). In the cross-ZC, which ZF was used as maternal parent and CC as paternal fish, there existed 93.3% fertilization rate and 35.67% hatching rate. By contrast, in the reverse cross-CZ, the fertilization rate of embryos was decreased to 79.4%, and no one could hatch (Table 1).

3.2. Observation of egg envelopes in hybrid and parents

Egg envelopes of the ZF, CC and their hybrids were observed by scanning electron microscopy. As shown in Fig. 2, the egg envelope of CC was about 4.80 μm (Fig. 2A and B), which was thicker than the ZF (about 0.80 μm) (Fig. 2C and D). Lots of circular holes uniformly distributed in the outer and near the outer layers of the egg envelope, but not existed in the inner layer (Fig. 2). We also noticed that some ridges were on the outer surface of egg envelope in CC (Fig. 2I), while no ridge in ZF (Fig. 2E). As for the hybrid offspring, the structure of egg envelopes were like their maternal fish (Figure 2M,N,Q,R).

We further observed the structure changes of egg envelope in the process of embryonic development. It showed that the inner surface of egg envelopes still maintained dense state in somatic pigment embryos of ZF, CC and their hybrids (Fig. 2H,I,P,T). And no obvious different in outer envelope were found between blastocyst and somatic pigmentation

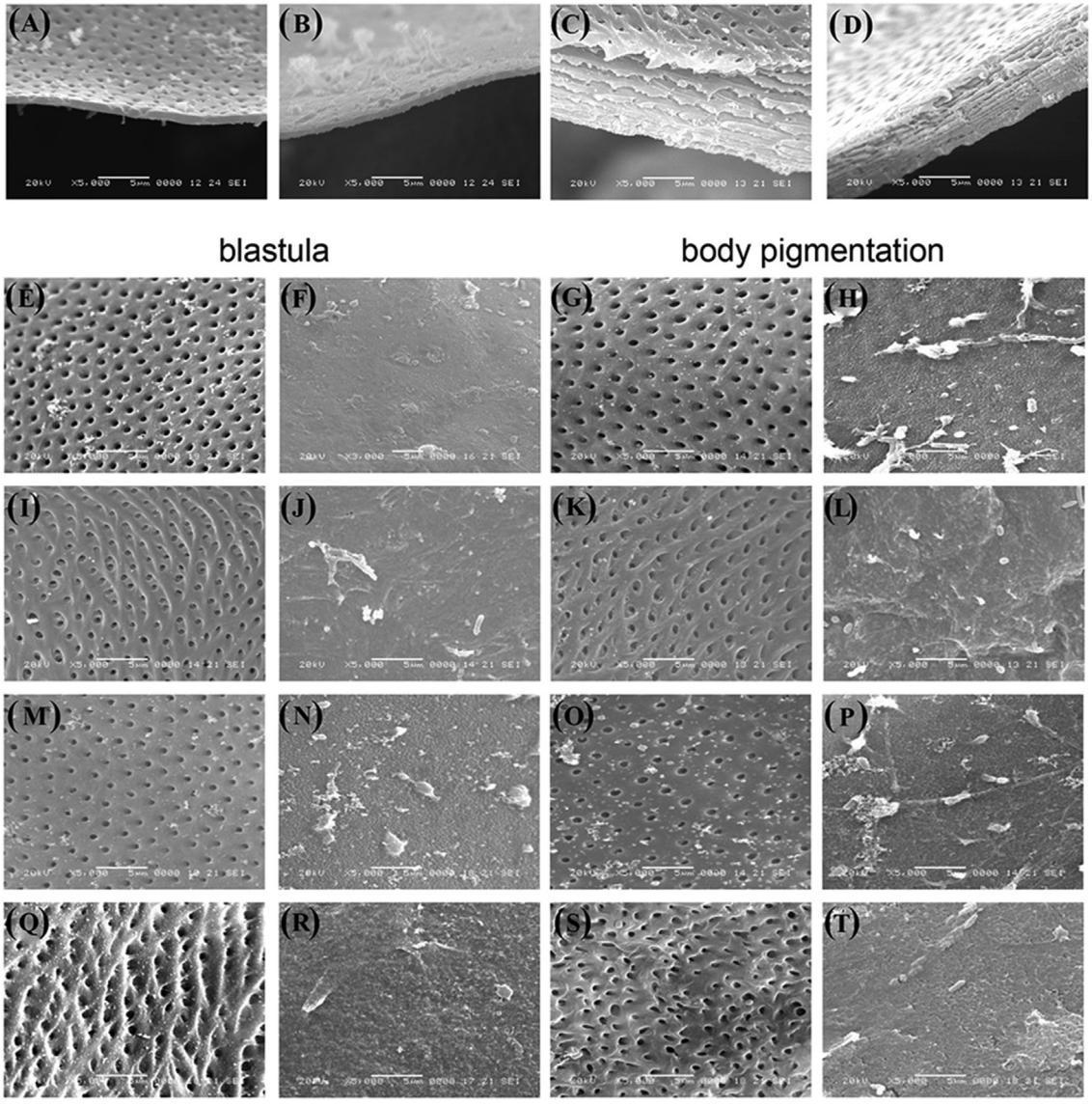

Fig. 2. Comparative observation of the egg envelope structure of ZF and CC and their hybrids by scanning electron microscopy. (A, B) Egg envelope of zebrafish; (C, D) egg envelope of crucian carp; egg envelope surface of ZF (E, F, G, H), CC (I, J, K, L), ZC (M, N, O, P) and CZ (Q, R, S, T), respectively.

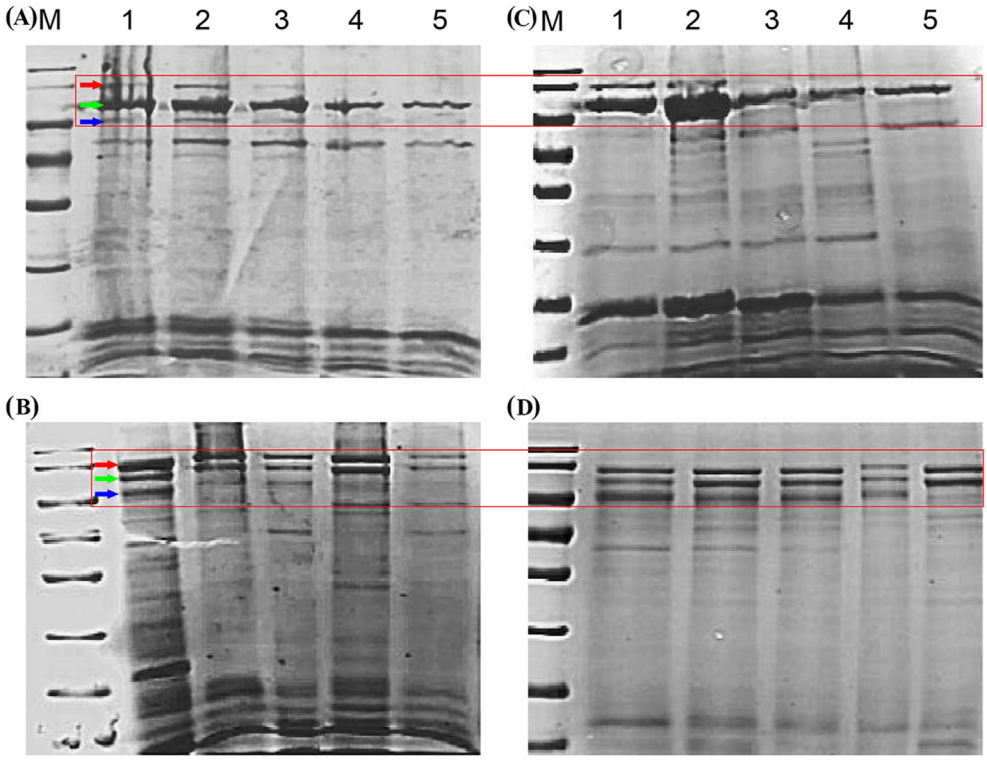

Fig. 3. SDS-PAGE patterns of egg envelope from ZF (A), CC (B), ZC (C), and CZ (D). The gel was stained with Coomassie Brilliant Blue G. "M" refers to the molecular markers. Lane 1 to 5 refers to egg envelopes from eggs at the stages of blastula, gastrula, optical vesicle, eye pigmentation, and body pigmentation, respectively. At the stage of blastula, the protein band 1 (b1, red arrow), band 2 (b2, green arrow) and band 3 (b3, blue arrow) were three major bands in the egg envelopes of CC and CZ, but only the b2 was high expression in the ZF and ZC.

embryos in ZF, CC and hybrid ZC (Fig. 2G, K, O), except there existed some deformations, such as the disappearance of ridges and irregular-shaped of holes in the CZ (Fig. 2S).

3.3. SDS-PAGE analysis of egg envelope in hybrids and their parent

Proteins of egg envelope were further analyzed in the hybrids and their parents by SDS-PAGE. As shown in Fig. 3, the SDS-PAGE patterns of the proteins in egg envelopes were existed obvious contrast between the ZF, CC (Fig. 3A and B). For example, at the stage of blastula, the protein band 1 (b1), band 2 (b2) and band 3 (b3) were three major bands with high expression levels in the egg envelopes of CC (Fig. 3B), but only the b2 was high level in the ZF (Fig. 3A). The protein profile of egg envelopes also changed in different stages of embryonic development (Fig. 3). In the ZC, the envelop protein patterns of different development stages embryos were similar to its maternal parent, not paternal parent (Fig. 3A, C). However, there existed significant differences between CZ and its maternal parent (CC) in the envelop protein patterns of different development stages embryos, e.g. in comparing with blastula, the levels of b3 were decreased obviously in the CC at the stages of gastrula, and optical vesicle, and eye pigmentation, however, in the CZ, there was litter change of b3 level among those different stage embryos (Fig. 3B, D).

3.4. Analysis of hatching enzyme gene cDNA and its physicochemical properties

In the ZF, two hatching enzyme homologues were reported, *zhe1* and *zhe2*, which *zhe1* was mainly expressed in pre-hatching embryos [24]. According to the *zh1* nucleotide sequence (GenBank No. NM_001045174), two *he1.1* oligo primers were designed (see Table S1) to clone the CDS of hatching enzyme gene from CC and hybrids. The hatching enzyme gene 1 (*he1*) CDS of CC was 786 bps coding for 261 amino acids (GenBank accession No. MH202659). As shown in Fig. 4A, the *he1* CDS nucleotide sequences from ZF and CC were 85.1% identity, while that of 99.6% identity was between the ZF and ZC, and 98.4% from CC and hybrid of CZ. Using ClustalW (https://www.genome.jp/tools-bin/clustalw), the sequence alignment analysis was shown that the amino acid residues of *he1* among hybrids and their parents, 57 were non-conservative, with a mutation rate of 22% (Fig. 4B).

Using the Protparam tool (http://web.expasy.org/proparam/), the protein physicochemical properties of *he1* in hybrids and their parents were analyzed. The relative molecular weight of *he1* in ZF and hybrid ZC were all 29448.44, their theoretical PI value was 6.52, and molecular formula was $C_{1309}H_{2057}N_{359}O_{396}S_9$. In the CC and hybrid CZ, the *he1*'s relative molecular weights, theoretical PI values and their molecular formulas were 29686.40 and 299771.33, 6.31 and 6.17, $C_{1303}H_{2031}N_{367}O_{404}S_{12}$ and $C_{1301}H_{2022}N_{372}O_{407}S_{12}$, respectively.

3.5. Expression characteristics of hatching enzyme gene in hybrids and parents

According to the ZF genome project, three orthologues were clustered in the genome, named *he1a*, *he1b* and *he2* [25]. To understand the expression characteristic of hatching enzyme gene in the distant hybridization offspring, we further analyzed the expression of three hatching enzyme genes (*he1a, he1b* and *he2*) at different development stages of embryo. All the primers used in this study were listed in Table S1. In ZF and the ZC, *he1a* and *he1b* genes were high expression in the embryos of muscle differentiation stage, then, were reduced significantly before hatching (Fig. 5A, C). However, in CC and the CZ, the expression levels of *he1a* and *he1b* gene were increased from gastrula to pre-hatching stages (Fig. 5B, D). We noted that the *he2* gene was only expressed in the ZF and ZC from optical vesicle stage to eye pigmentation stage embryo, but not expressed in embryos of the CC and CZ (Fig. 5).

To further identify the expression characteristics of *he1* gene, we constructed a vector named p-HE-EGFP-N1 plasmid, and the expression of *he1* was enhanced by CMV promoter (Fig. 6A). As shown in Fig. 6C, EGFP was appeared obviously at the neuro-embryo stage, then, were increased gradually with embryo's development. After hatching, the fluorescence could be seen in the ventral yolk of the fry (Fig. 6F,K). These expression characteristics of *he1* gene in CC were consistent with reports in other fish species [26,27].

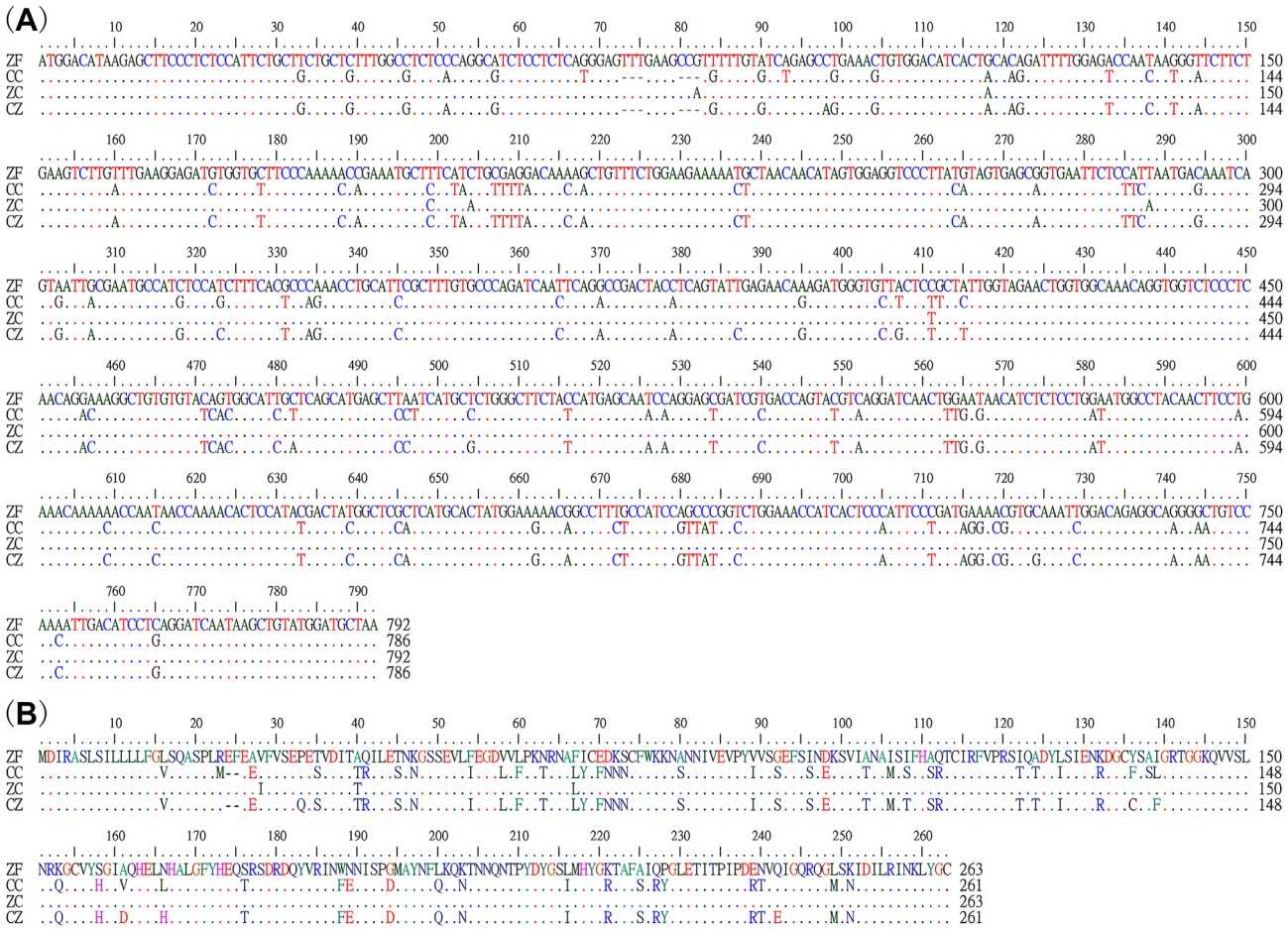

Fig. 4. Multiple nucleotide sequence (A) and amino acid sequence alignment (B). Comparison of the CDS nucleotide sequences and amino acid sequences *he1* gene of ZF, CC, ZC, and CZ. Sequence alignment of hatching enzyme protein using the ClustalW, each color represents a class of amino acid residues with similar structure and function.

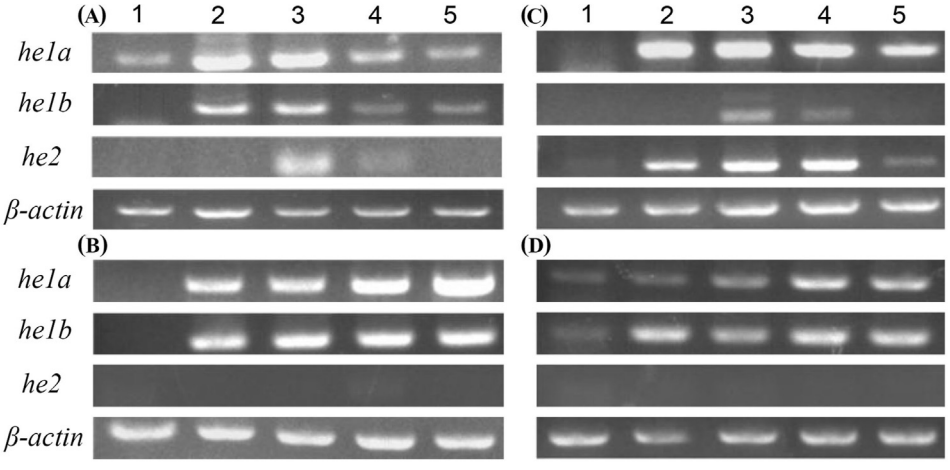

Fig. 5. RNA expression patterns of hatching enzyme gene from ZF (A), CC (B), ZC (C), and CZ (D). Lane 1 to 5 refers to embryos at the stages of gastrula, optical vesicle, muscle differentiation, eye pigmentation, and body pigmentation.

4. Discussion

In this study, cross-breeding between zebrafish (ZF) and crucian carp (CC) were generated with low hatchability, in especial in hybrid progenies from Crucian carp♀ × Zebrafish♂(Fig. 1). Our results showed that the characteristics of egg envelope in hybrid progenies were inherited from maternal fish, while there were obvious differences in predictive protein physicochemical properties of *he1* between the CZ and CC. During the process of embryo development, we also founded some obvious differences between the CZ and CC, not only in the structures and SDS-PAGE protein pattern of egg envelopes, but also *he1* mRNA levels (Figs. 2, 3 and 5). Hatching enzyme, secreted by hatching gland cells, is

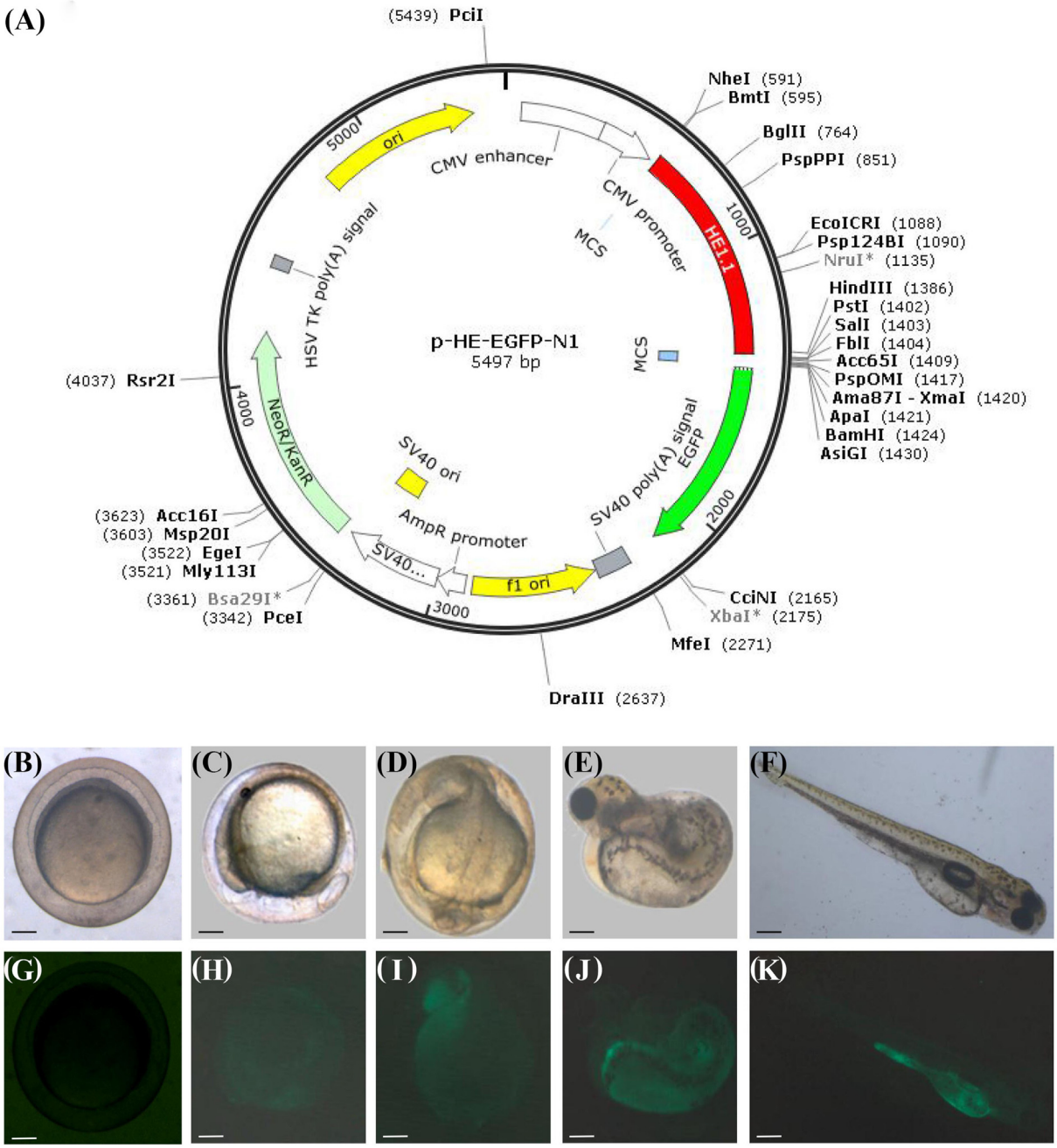

Fig. 6. Expression of EGFP-labeled hatching enzyme in different stage embryo of CC. (A) showed the construct of p-HE-EGFP-N1 plasmid. Embryos were observed at the stages of neurula (C,H), optical vesicle (D,I), body pigmentation (E, J), and hatched fry (F,K). (B, G) showed the control group at stage of somite. The scale bar = 0.3 mm.

one of proteolytic enzymes determining embryo hatching [36-41]. In medaka, it has reported that two kinds of hatching enzyme have been identified, named the high choriolytic enzyme (HCE) and low choriolytic enzyme (LCE) [43,44]. The HCE can swell the egg envelope by its proteolytic action [43,45], while the LCE effectively digest the swollen inner layer of egg envelope under pretreatment of HCE. Therefore, during the hatching, the egg envelope inner layer effectively digest by the cooperation of HCE and LCE [46]. Two hatching enzyme homologues, ZHE1 and ZHE2, were reported in the zebrafish, which ZHE1 was identified as an ancestral hatching enzyme [24,42]. In our data, RT-PCR analysis was revealed that *he1a* and *he1b* were highly expressed in embryos of ZF, CC, and hybrids. The *he2* was rarely expressed in pre-hatching embryos, but was expressed in CC embryos at stage of muscle differentiation, and in CZ embryos at stages of optical vesicle, muscle differentiation, and eye pigmentation (Fig. 5). It is considered that the hatching of zebrafish embryo was performed by a single enzyme [24]. Therefore, the biological function of HE2 needs further study.

Furthermore, fish egg envelope is the outer proteinaceous layer of fish [28]. The morphological structure of egg envelope could be not only used to distinguish fish family or genus [29,30], but also directly related to the stability of the environment within the embryonic development, such as protecting or fixing the embryo body [31], material and even gas exchanging can be carried out in the pores on the surface of the egg envelope [32,33], and participating in the fertilization process [34]. Our previous report also showed that egg envelopes of CC had strong ability to block Cd and could protect embryos from Cd stress damage [35]. In this study, there were obvious differences between ZF and CC in the characteristics of egg envelope (Figs. 2 and 3). We noticed that the inner

egg envelope maintained a dense structure, which is considered to maximize the stability of the internal environment of embryo and could ensure embryo safety. Our results also showed that there existed obvious differences in the outer surface structure of egg envelope between CC and ZF (Fig. 2). We considered that the non-smooth outer surface of egg envelope might be related to the characteristics of adhesive egg in CC, while the smooth surface of ZF egg envelope adapts to its non-adhesive egg.

In summary, in this study, we furtherly demonstrated that the egg envelope and hatching enzyme in hybrid progenies were inherited from the maternal fish, and thought that the low hatchability of CC♀ × ZF♂ might be due to the changes of *he1* gene expression levels and its protein physicochemical properties in cross progenies. These results provide insights for further establishing the strategies of distant hybridization breeding.

Author contributions

YX, LP, and WF designed the experiments. WF, JZ, WX, SH and ML carried out the experiments. WL, JL, WF, and YX conducted the statistical analysis and discussion. WF and YX organized and wrote the manuscript. All the authors have read the paper and agreed to list their names as coauthors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2018YFD0901202), and the Hunan Provincial Natural Science and Technology Major Project (2017NK1031).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.repbre.2021.04.001.

Funding

This work was supported by the National Key Research and Development Program of China (2018YFD0901202), the National Science Foundation of China (31772902), and the Hunan Provincial Natural Science and Technology Major Project (2017NK1031).

References

[1] M. Beck, C. Biggers, H. Dupree, Karyological analysis of Hypophthalmichthys molitrix, Aristichthys nobilis and their F1 hybrid, Trans. Am. Fish. Soc. 109 (1980) 433–438.
[2] Y.D. Lou, X.Q. Li, Distant hybridization of fish and its application in aquaculture in China, J. Fish. Sci. China 13 (2006) 151–158.
[3] J. Hu, S. Liu, J. Xiao, Y. Zhou, C. You, W. He, R. Zhao, C. Song, Y. Liu, Characteristics of diploid and triploid hybrids derived from female Megalobrama amblycephala Yih × male Xenocypris davidi Bleeker, Aquaculture 364 (2012) 157–164.
[4] J. Sui, Q. Liu, Z. Xiao, D. Ma, S. Xu, T. He, Y. Liu, Y. Xiao, J. Li, The viability, melanophore and embryo genesis of first- and second-generation hybrids between Paralichthys olivaceus and P. dentatus, Mar. Biol. Res. 9 (2) (2013) 220–226.
[5] Q. Qin, Y. Wang, J. Wang, J. Dai, J. Xiao, F. Hu, Y. Liu, The autotetraploid fish derived from hybridization of Carassius auratus red var.(female)× Megalobrama amblycephala (male), Biol. Reprod. 91 (4) (2014) 91–93.
[6] Z.H. Zhang, J. Chen, L. Li, M. Tao, S.J. Liu, Research advances in animal distant hybridization, Sci. China Life Sci. 57 (9) (2014) 889–902.
[7] L. Yang, T. Sado, M.V. Hirt, E. Pasco-Viel, M. Arunachalam, J. Li, A.M. Simons, Phylogeny and polyploidy: resolving the classification of cyprinine fishes (Teleostei: cypriniformes), Mol. Phylogenet. Evol. 85 (2015) 97–116.
[8] A.J. Kendall, Early life history stages of fishes and their characters, Ontogeny and systematics of fishes 1 (1984) 11–22.
[9] S. Lönning, Comparative electron microscopic studies of teleostean eggs with special reference to the chorion, Sarsia 49 (1) (1972) 41–48.
[10] E. Anderson, The formation of the primary envelope during oocyte differentiation in teleosts, JCB (J. Cell Biol.) 35 (1) (1967) 193–212.
[11] J.N. Dumont, A.R. Brummet, The vitelline envelope, chorion, and micropyle of Fundulus heteroclitus eggs, Gamete Res. 3 (1) (1980) 25–44.
[12] J.P. Wourms, Annual fish oogenesis. I. Differentiation of the mature oocyte and formation of the primary envelope, Dev. Biol. 50 (2) (1976) 338–354.
[13] F. Tingjun, S. Zhenping, Advances and prospect in fish hatching enzyme research, Trans. Oceanol. Limnol. 1 (2002) 48–56.
[14] J. Ishida, Hatching enzyme : past, present and future, Zool. Sci. 2 (1985) 1–10.
[15] A.M. Schoots, R.C. Meijer, J.M. Denucé, Dopaminergic regulation of hatching in fish embryos, Dev. Biol. 100 (1) (1983) 59–63.
[16] K. Yamagami, Mechanisms of hatching in fish: secretion of hatching enzyme and enzymatic choriolysis, Integr. Comp. Biol. 21 (2) (1981) 459–471.
[17] J. Hiroi, K. Maruyama, K. Kawazu, T. Kaneko, R. Ohtani-Kaneko, S. Yasumasu, Structure and developmental expression of hatching enzyme genes of the Japanese eel Anguilla japonica: an aspect of the evolution of fish hatching enzyme gene, Dev. Gene. Evol. 214 (4) (2004) 176–184.
[18] Kenjiro Yamagami, Mechanisms of hatching in fish, in: Fish Physiology, vol. 11, 1988, pp. 447–499.
[19] Katagiri Chiaki, Properties of the hatching enzyme from frog embryos, J. Exp. Zool. 193 (1) (1975) 109–118.
[20] G. Jianfang, Z. Li, Genetic basis and breeding application of clonal diversity and dual reproduction modes in polyploid Carassius auratus gibelio, Sci. China Life Sci. 53 (4) (2010) 409–415.
[21] S. Wang, C. Tang, M. Tao, Q. Qin, C. Zhang, K. Luo, J. Xiao, Establishment and application of distant hybridization technology in fish, Sci. China Life Sci. 62 (1) (2019) 22–45.
[22] J. Wang, J. Xiao, M. Zeng, K. Xu, M. Tao, C. Zhang, Y. Liu, Genomic variation in the hybrids of white crucian carp and red crucian carp: evidence from ribosomal DNA, Sci. China Life Sci. 58 (6) (2015) 590.
[23] K.U. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (5259) (1970) 680–685.
[24] K. Sano, K. Inohaya, M. Kawaguchi, N. Yoshizaki, I. Iuchi, S. Yasumasu, Purification and characterization of zebrafish hatching enzyme – an evolutionary aspect of the mechanism of egg envelope digestion, FEBS J. 275 (23) (2008) 5934–5946.
[25] M. Kawaguchi, S. Yasumasu, J. Hiroi, K. Naruse, T. Suzuki, I. Iuchi, Analysis of the exon-intron structures of fish, amphibian, bird and mammalian hatching enzyme genes, with special reference to the intron loss evolution of hatching enzyme genes in Teleostei, Gene 392 (1–2) (2007) 77–88.
[26] K. Inohaya, S. Yasumasu, M. Ishimaru, A. Ohyama, I. Iuchi, K. Yamagami, Temporal and spatial patterns of gene expression for the hatching enzyme in the teleost embryo, Oryzias latipes, Dev. Biol. 171 (2) (1995) 374–385.
[27] M. Kawaguchi, S. Yasumasu, J. Hiroi, K. Naruse, M. Inoue, I. Iuchi, Evolution of teleostean hatching enzyme genes and their paralogous genes, Dev. Gene. Evol. 216 (12) (2006) 769–784.
[28] V.A. Iconomidou, D.G. Chryssikos, V. Gionis, M.A. Pavlidis, A. Paipetis, S.J. Hamodrakas, Secondary structure of chorion proteins of the teleostean fish Dentex dentex by ATR FT-IR and FT-Raman spectroscopy, J. Struct. Biol. 132 (2) (2000) 112–122.
[29] N.R. Merrett, S.H. Barnes, Preliminary survey of egg envelope morphology in the Macrouridae and the possible implications of its ornamentation, J. Fish. Biol. 48 (1) (1996) 101–119.
[30] E. Rizzo, Y. Sato, B. Barreto, H. Godinho, Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts, J. Fish. Biol. 61 (3) (2002) 615–632.
[31] C.M. Stehr, J.W. Hawkes, The comparative ultrastructure of the egg membrane and associated pore structures in the starry flounder, Platichthys stellatus (Pallas), and pink salmon, Oncorhynchus gorbuscha (Walbaum), Cell Tissue Res. 202 (3) (1979) 347–356.
[32] A.M. Fausto, S. Picchietti, A.R. Taddei, C. Zeni, G. Scapigliati, M. Mazzini, L. Abelli, Formation of the egg envelope of a teleost, Dicentrarchus labrax (L.): immunochemical and cytochemical detection of multiple components, Anat. Embryol. 208 (1) (2004) 43–53.
[33] S. Kudo, Ultrastructure and Ultracytochemistry of Fertilization Envelope Formation in the Carp Egg : (vitelline envelope/fertilization envelope/ultrastructure/ultracytochemistry/carp egg), Dev. Growth Differ. 24 (4) (1982) 327–339.

[34] S. Otani, T. Iwai, S. Nakahata, C. Sakai, M. Yamashita, Artificial fertilization by intracytoplasmic sperm injection in a teleost fish, the medaka (Oryzias latipes), Biol. Reprod. 80 (1) (2009) 175–183.
[35] W. Liu, Y. Wen, M. Wang, S. Gui, X. Li, Y. Fan, J. Liu, Enhanced resistance of triploid crucian carp to cadmiuminduced oxidative and endoplasmic reticulum stresses, Curr. Mol. Med. 18 (6) (2018) 400–408.
[36] J. Ishida, Hatching enzyme in the fresh-water fish,Oryzias latipes, Annot. Zool. Jpn. 22 (1944) 137–164.
[37] C. Katagiri, R. Maeda, C. Yamashika, K. Mita, S. Yasumasu, Molecular cloning of Xenopus hatching enzyme and its specific expression in hatching gland cells, Int. J. Dev. Biol. 41 (1) (1997) 19–25.
[38] S. Yasumasu, K.M. Mao, F. Sultana, H. Sakaguchi, N. Yoshizaki, Cloning of a quail homologue of hatching enzyme: its conserved function and additional function in egg envelope digestion, Dev. Gene. Evol. 215 (10) (2005) 489–498.
[39] J.S. Bond, R.J. Beynon, The astacin family of metalloendopeptidases, Protein Sci. 4 (7) (1995) 1247–1261.
[40] V. Quesada, L.M. Sanchez, J. Alvarez, C. Lopez-Otin, Identification and characterization of human and mouse ovastacin: a novel metalloproteinase similar to hatching enzymes from arthropods, birds, amphibians, and fish, J. Biol. Chem. 279 (25) (2004) 26627.
[41] K.Y. Yamagami, T.S. Hamazaki, S. Yasumasu, K. Masuda, I. Iuchi, Molecular and cellular basis of formation, hardening, and breakdown of the egg envelope in fish, Int. Rev. Cytol. 136 (1992) 51–92.
[42] S. Lin, Y. Zhao, Z. Ji, J. Ear, C.H. Chang, H. Zhang, C. Low-Kam, K. Yamada, H. Meng, X. Wang, Zebrafish high-throughput screening to study the impact of dissolvable metal oxide nanoparticles on the hatching enzyme, ZHE1. Small. 9 (9–10) (2013) 1776–1785.
[43] S. Yasumasu, I. Iuchi, K. Yamagami, Purification and partial characterization of high choriolytic enzyme (HCE), a component of the hatching enzyme of the teleost, Oryzias latipes, J. Biochem. 105 (2) (1989) 204–211.
[44] S. Yasumasu, I. Iuchi, K. Yamagami, Isolation and some properties of low choriolytic enzyme (LCE), a component of the hatching enzyme of the teleost, Oryzias latipes, J. Biochem. 105 (2) (1989) 212–218.
[45] K.S. Lee, S. Yasumasu, K. Nomura, I. Iuchi, HCE, a constituent of the hatching enzymes of Oryzias latipes embryos, releases unique proline-rich polypeptides from its natural substrate, the hardened chorion, FEBS Lett. 339 (3) (1994) 281.
[46] S. Yasumasu, H. Shimada, K. Inohaya, K. Yamazaki, I. Iuchi, I. Yasumasu, K. Yamagami, Different exon–intron organizations of the genes for two astacin-like proteases, high choriolytic enzyme (choriolysin H) and low choriolytic enzyme (choriolysin L), the constituents of the fish hatching enzyme, Eur. J. Biochem. 237 (3) (1996) 752–758.