Comparison of the expression patterns of several sox genes between *Oryzias latipes* and *Danio rerio*

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High mobility group domain containing transcription factors “sox” have a variety of functions in Metazoans such as formation of the ectoderm and development of the neural system (Sasai, 2001). They may behave as either activator or repressor for the genes which are regulating these functions (Chew et al., 2009). The sox genes are grouped into 11 subfamilies according to their phylogenetic relationship (Cui et al., 2011). The group B sox genes are especially important for the neural development since they are affective on the formation of the central neural system, formation of the sensory organs (such as eyes) and so on (Kamachi et al., 2009).

The aim of this project was the observation and the comparison of some B group sox genes’ expression patterns during development of the medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*).

Introduction:

The gene expression of the sox transcription factors is important for the development of human and many other chordates including mouse and fish. They are especially important for the neural development and germ line maturation (Schepers et al., 2002). The sox genes which belong to the same subfamilies show similarities with one another in terms of their sequences and functions during the development (Wegner, 2010). Genes sox2, sox3, sox14 and sox21 are all belong to the same subfamily: they are all group B sox genes. Group B type sox genes are also divided into 2 groups as B1 group sox genes and B2 group sox genes. The genes sox2 and sox3 belong to B1 group while sox14 and sox21 belong to B2 group. Although they have similar biochemical properties and functions during the development, B1 group sox genes act as activators and B2 group sox genes act as repressors (Cui et al., 2011).

The aim of the project was to compare expression patterns of several sox genes (sox2, sox3, sox14 and sox21) between medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*). It has been known that sox2 is expressed in the anterior endoderm during the developmental process in the mammals (Que et al., 2007). The main reason was to investigate whether the sox2 gene is expressed also in the anterior endoderm of medaka and zebrafish. To examine it clearly, since sox2 gene belongs to group B sox genes, 3 other group B sox genes were investigated along with sox2 gene.

As the current knowledge of the sox expression patterns in medaka and zebrafish states, sox2 is expressed to be found in the brain in medaka (Cui et al., 2011) and in the presumptive spinal cord and forebrain in the zebrafish (Okuda et al., 2006). sox3 gene expression is seen in the neuroectoderm and in the sensory organs, especially in the eyes, in medaka (Köster et al., 2000) and in the spinal cord, hindbrain and forebrain in zebrafish (Okuda et al., 2006). Expression of sox21 is seen in the eyes, brain, sexual organs and intestine (Cui et al., 2011) in the medaka. Since it is duplicated in zebrafish, there are two genes corresponding to sox21 in zebrafish: sox21a and sox21b. sox21a is expressed in the brain, ovary and intestine and sox21b is expressed in the testis and brain (Lan et al., 2011). There are plenty of information about these B group sox genes except sox14. sox14 expression pattern has not been shown yet neither in medaka nor in zebrafish.
Methods:

Wild type medaka and zebrafish embryos were collected and let grow until they reach to desired developmental stage.

Collected embryos were fixed in PFA at different stages (Medaka embryos were fixed at stage 25 and stage 32; zebrafish embryos were fixed at 21-somites stage and pecfin stage.). All of the early stage embryos (medaka stage 25 and zebrafish 21-somites stage) and half of the late stage embryos (medaka stage 32 and zebrafish pecfin stage) were prehybridized prior to Whole-Mount In situ Hybridization.

RNA Extraction with ISOGEN was done with the remaining half amount of the collected late stage embryos.

cDNA was synthesized from the extracted RNA with the primers designed to obtain the desired sox genes (4 primer sets for medaka and 5 primer sets for zebrafish).

cDNA was cloned (according to the Invitrogen TOPO TA Cloning procedure) and transformation into Escherichia coli (E.coli) DH5α cells was done. Blue/white selection of the colonies was done via LacZ system. White colonies were chosen for colony PCR. Cultures were prepared from desired colonies.

Upon midipreparation of the cultures (according to the QIAprep Miniprep Kit protocol), the orientation of the cloned cDNA sequences was determined by sequencing (according to the Big Dye Sequencing procedure).

Labelled RNA probe was synthesized (according to Whole-Mount Single Color In situ Hybridization protocol).

Whole-Mount In situ Hybridization was performed according to its protocol. Color reaction was observed and the results were examined under microscope (see the results in the Results section).

Results:

Figure 1: sox14 gene expression in early stage fish embryos from dorsal (up: anterior) and lateral (left: anterior) views. A: dorsal (a) and lateral (b) views of stage 25 medaka embryos. B: dorsal (c) and lateral (d) views of 21-somites stage zebrafish embryos.
**Figure 2:** *sox14* gene expression in late stage fish embryos from dorsal (up: anterior) and lateral (left: anterior) views. **A:** dorsal (a) and lateral (b) views of stage 32 medaka embryos. **B:** dorsal (c) and lateral (d) views of pcfin stage zebrafish embryos.

**Figure 3:** *sox2* gene expression in early stage fish embryos from dorsal (up: anterior) and lateral (left: anterior) views. **A:** dorsal (a) and lateral (b) views of stage 25 medaka embryos. **B:** dorsal (c) and lateral (d) views of 21-somites stage zebrafish embryos.
Figure 4: sox2 gene expression in late stage fish embryos from dorsal (up: anterior) and lateral (left: anterior) views. A: dorsal (a) and lateral (b) views of stage 32 medaka embryos. B: dorsal (c) and lateral (d) views of pecfin stage zebrafish embryos.

Figure 5: sox2 gene expression around the anterior endoderm regions of late stage fish embryos from the lateral (left: anterior) view. A: view of stage 32 medaka embryo. B: view of pecfin stage zebrafish embryo.
Figure 6: *sox3* gene expression in medaka embryos from dorsal (up: anterior) and lateral (left: anterior) views. **A:** dorsal (a) and lateral (b) views of stage 25 medaka embryos. **B:** dorsal (c) and lateral (d) views of stage 32 medaka embryos.

Figure 7: *sox3* gene expression comparison between early stage medaka and zebrafish embryos from the dorsal (up: anterior) view. **A:** view of stage 25 medaka embryo. **B:** view of 21-somites stage zebrafish embryo.

_taken from Okuda et al. (2006)_

(mhb: midbrain-hindbrain boundary, le: lens, olp: olfactory placode)
**Figure 8:** *sox21b* gene expression in zebrafish embryos from dorsal (up: anterior) and lateral (left: anterior) views. **A:** dorsal (a) and lateral (b) views of 21-somites stage zebrafish embryos. **B:** dorsal (c) and lateral (d) views of pecfin stage zebrafish embryos.

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<th><em>sox2</em></th>
<th><em>sox3</em></th>
<th><em>sox14</em></th>
<th><em>sox21</em> (Medaka)</th>
<th><em>sox21a</em> (Zebrafish)</th>
<th><em>sox21b</em> (Zebrafish)</th>
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<tr>
<td><strong>Medaka</strong></td>
<td>Anterior brain, <em>notochord</em>, anterior endoderm</td>
<td>Brain, lens, midbrain-hindbrain boundary, olfactory placode</td>
<td>Brain, <em>neural tube</em></td>
<td><em>Not successful</em></td>
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<tr>
<td><strong>Zebrafish</strong></td>
<td>Anterior brain, <em>hindbrain</em>, anterior endoderm</td>
<td><em>Not successful</em></td>
<td>Hindbrain</td>
<td><em>Not successful</em></td>
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**Table 1:** Comparison of in situ hybridization results.

**Discussion:** While performing the project, it was aimed to find the expression patterns of *sox2*, *sox3*, *sox14* and *sox21* genes similar to expected patterns and to see *sox2* gene’s expression in the anterior endoderm of both medaka and zebrafish. As Table 1 states, in situ hybridization procedure was not successful for some samples. Results were failed to obtain
from sox21 samples of medaka and from sox3 and sox21a samples of zebrafish; since the transformations of these constructs into E.coli cells were not efficient enough. However, results were obtained from the other constructs. As it can be seen in Table 1, comparable results were obtained especially from sox2 and sox14 constructs of both medaka and zebrafish.

It was important to have the sox14 expression pattern results for both species since the expression pattern of sox14 was shown for neither medaka nor zebrafish. Figure 1 shows sox14 gene expression in medaka (a-b) and zebrafish (c-d). There is sox14 expression in the most parts of the brain of early stage medaka as the red arrow head indicates in the figures 1a and 1b. However, there is no expression observed in the early stage zebrafish embryos (figures 1c and 1d). As for the sox14 gene expression pattern of late stage embryos (Figure 2), there is a clear expression pattern in the brain (indicated with red arrow head) and neural tube (indicated with blue arrow head) of the medaka (figures 2a and 2b). The expression pattern of sox14 in late stage zebrafish embryos is not as certain and well-defined as in medaka, but expression can be seen in the hindbrain region (as indicated with the white arrow head) of the embryos (figures 2c and 2d). We can say that expression level is somehow higher in the medaka at that stage. When we compare early stage expressions (Figure 1) with late stage expressions (Figure 2), we can say that the expression level of sox14 increases in the late stages in both species and boundaries of the expression patterns can be detected easier in the late stage embryos.

As for sox2 early stage embryos (Figure 3), gene expression can be seen in the forebrain (indicated with red arrow head) of medaka (figures 3a and 3b) and in both forebrain and hindbrain (shown with orange arrow head) of zebrafish (figures 3c and 3d). As like in sox14 gene expression, the expression is not dense in the early stage embryos. Clearer expression pattern of sox2 gene is observed in late stage embryos (Figure 4). Figures 4a and 4b show the expression pattern of sox2 gene in late stage medaka embryos and figures 4c and 4d show the pattern in late stage zebrafish ones. In both fish, expression is observed in the anterior parts of the brain (shown with black arrow head) clearly compared to early stage ones. Moreover, expression was observed in the anterior endoderm parts of the embryos both in medaka and in zebrafish (shown with red arrow head in Figure 4 and Figure 5). Figure 5 is the magnified version of these parts of the fish from Figure 4. sox2 gene expression is seen in the anterior endoderm. It was known that sox2 is expressed in the anterior endoderm of the mammals and this results show that it is expressed in the same part in medaka and zebrafish, too.

The expression pattern of the sox3 gene was able to be observed only in medaka both in early and late stage embryos (Figure 6). Its expression seems denser in the early stages. Lenses (indicated with red arrow head) and notochord (shown with orange arrow head) are observed both in early and late stage medaka embryos. Midbrain-hindbrain boundary can also be observed in the early stage embryos as it is indicated with white arrow head in Figure 6b. Expression density of sox3 gene decreases in the late stages (figures 6c and 6d). sox3 gene expression pattern of 21-somites stage zebrafish embryos was shown previously in the paper of Okuda et al. (2006). Since 21-somites stage is the stage that was chosen as the early stage experiments in my project and I compare my results from medaka and zebrafish considering their stages, I compared the early stage medaka findings with the result from the paper of Okuda et al. (2006). Figure 7a shows the sox3 gene expression pattern in medaka observed by me and Figure 7b shows the sox3 expression pattern in zebrafish observed by the authors of that paper. As it can be seen, lenses, midbrain-hindbrain boundary and olfactory placode are observed in both species in the early stage.

Figure 8 shows the sox21b expression pattern in zebrafish. As it is seen in figures 8a and 8b, there is no sox21b expression in the early stage embryos. However, there is low
expression of sox21b around hindbrain in the later stages (shown with red arrow head in figures 8c and 8d).

Differences in the expression patterns of different species were seen in that project. There can be two main reasons for these results. One is that the genes may have different functions in medaka and zebrafish since they are two separate species. The other one is that the chosen stages might not be similar enough to compare the species. The stages were chosen considering the developmental similarities and the number of somites of the embryo. However, since the rate of development differs in these two species, it was not efficient enough to compare them.

**Summary:** To sum up, expression pattern of sox14 was shown for the first time both in medaka and zebrafish. According to the previous knowledge, it was known that sox2 is expressed in the anterior endoderm in mammals, experimental results of sox2 shows that it is expressed in medaka and zebrafish, too. As for sox3, the expression in medaka is similar to the current knowledge of the expression of that gene in zebrafish (stated by Okuda et al. (2006)). Finally, low level of sox21 expression was observed in zebrafish.
References:


