

Supplementary information

Retinoic Acid and Meiosis Induction in Adult versus Embryonic Gonads of Medaka

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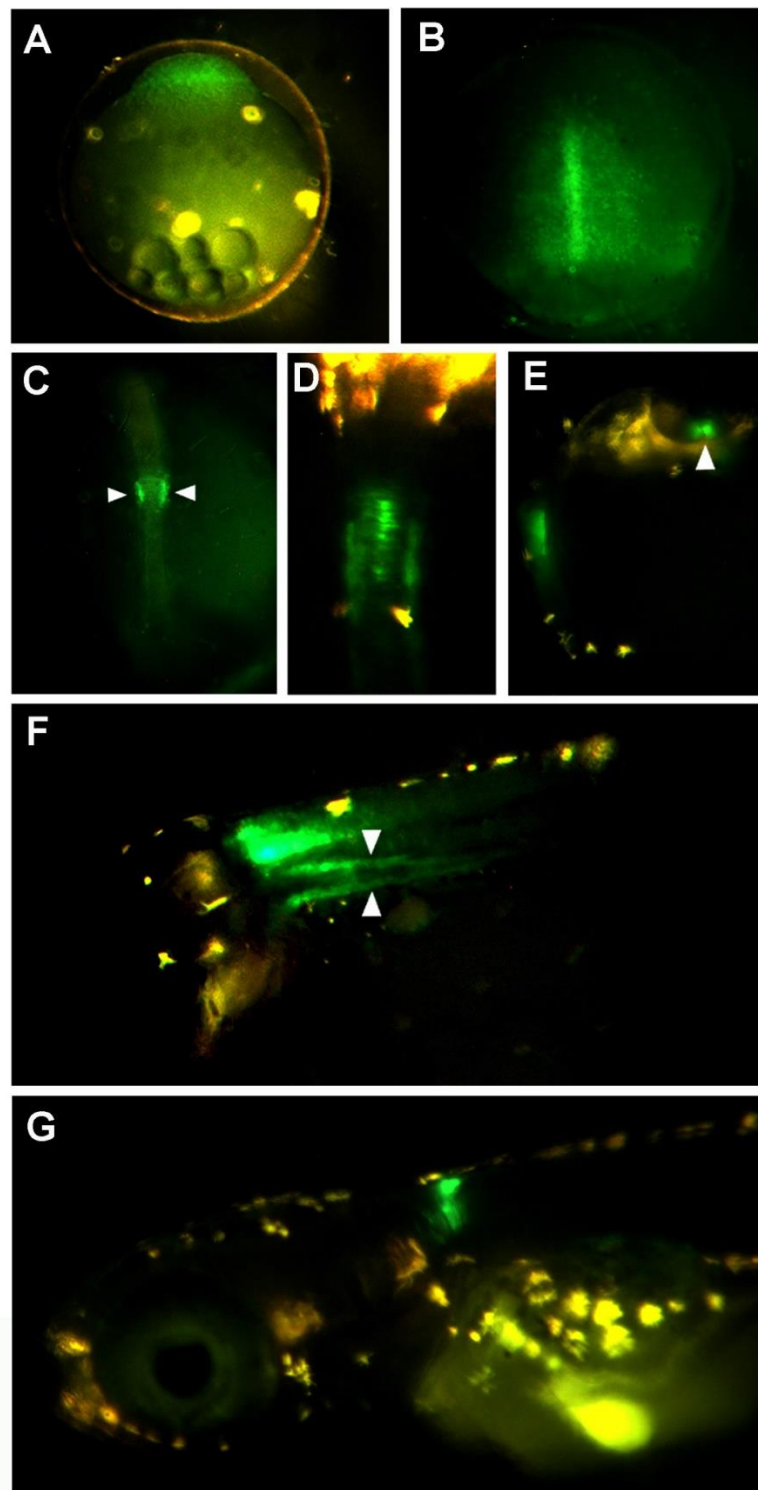
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SUPPLEMENTARY TABLE

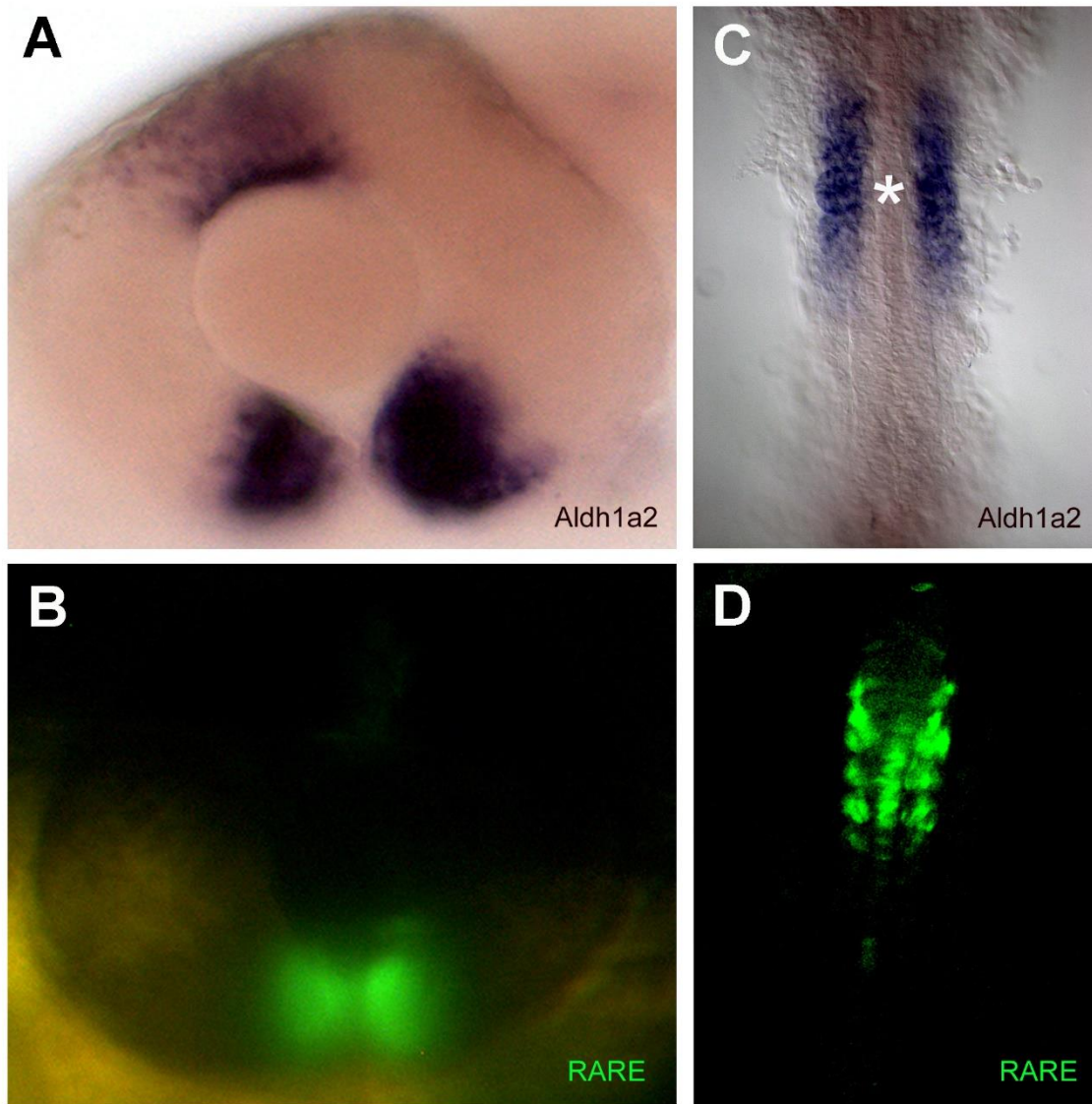
Primer	Sequence	Purpose
Aldh1a2_RT_F	5'-GCCTTCTCTTTGGGCTCAGT-3'	qRT-PCR
Aldh1a2_RT_R	5'-GGAAATGCGGTGTAGTGGTC-3'	qRT-PCR
Cyp26a1_RT_F	5'-AGAGATGAAGCGGCTGATGT-3'	qRT-PCR
Cyp26a1_RT_R	5'-TCTTCTTGCTGCTGTCGATG-3'	qRT-PCR
Cyp26b1_RT_F	5'-ATCCGAGCACGAGACATCTT-3'	qRT-PCR
Cyp26b1_RT_R	5'-GGATGTTGGAGGAGTTGCAT-3'	qRT-PCR
Cyp26c1_RT_F	5'-CAGAGGGGCTTGACTTTGAC-3'	qRT-PCR
Cyp26c1_RT_R	5'-CAGCCTTTGGGAATCTGGTA-3'	qRT-PCR
Dmc1-RT-F	5'-GTTTCCAGACGGCCTTTGAG-3'	qRT-PCR
Dmc1-RT-R	5'-CGATGTCTCTCAGCCTGTCT-3'	qRT-PCR
Efla-RT-F	5'-GCCCCTGGACACAGAGACTTCATCA-3'	qRT-PCR
Efla-RT-R	5'-AAGGGGGCTCGGTGGAGTCCAT-3'	qRT-PCR
Aldh1a2_Probe_F	5'-CAGAGTGGAATCCAGGAAGG-3'	<i>in situ</i> hybridization probe
Aldh1a2_Probe_R	5'-GGAAATGCGGTGTAGTGGTC-3'	<i>in situ</i> hybridization probe
Cyp26a1_Probe_F	5'-CTTTCAGCTCAATGGCTTCC-3'	<i>in situ</i> hybridization probe
Cyp26a1_Probe_R	5'-CAAACGTCATGAGCAGCCTA-3'	<i>in situ</i> hybridization probe

Supplementary Table 1 –Sequence of primers used in the present study

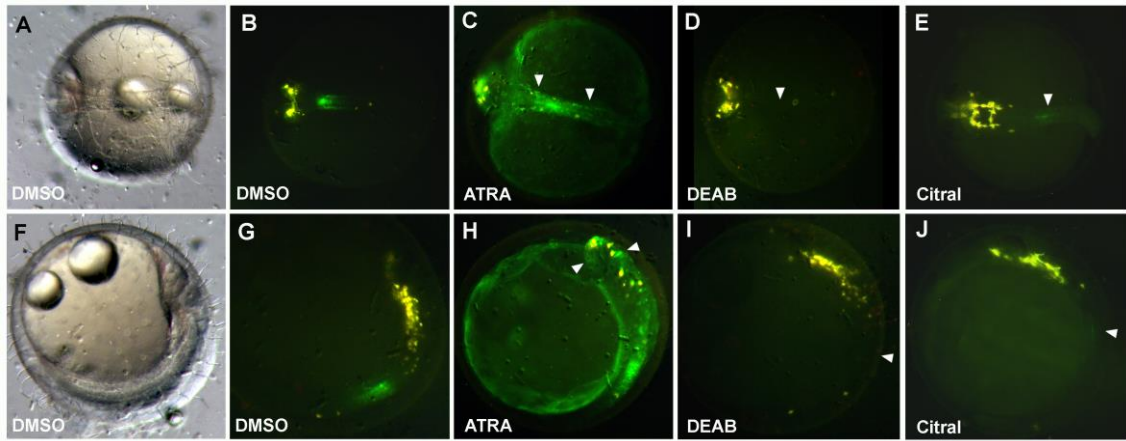
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Spatial and temporal expression patterns of 12X Retinoc Acid Responsive Element (RARE) transgenic line during medaka development. (A) Early blastula stage with ubiquitous RA-responsiveness. (B) Late gastrula stage with ubiquitous responsiveness on the embryonic shield. (C) Stage 18 with responsiveness on the lateral plate mesoderm (arrow head). (D) Stage 23 (12 somite stage) responsiveness in the somites (until 5-6), neural tube and notochord. (E) Stage 27 showing responsiveness in the retina, strongly at the ventral part (arrow head). (F) Stage 33 with pronephros responsiveness clearly recognizable (arrow heads). (G) Hatching stage, with responsiveness of the hindbrain being more prominent compared to other tissues.

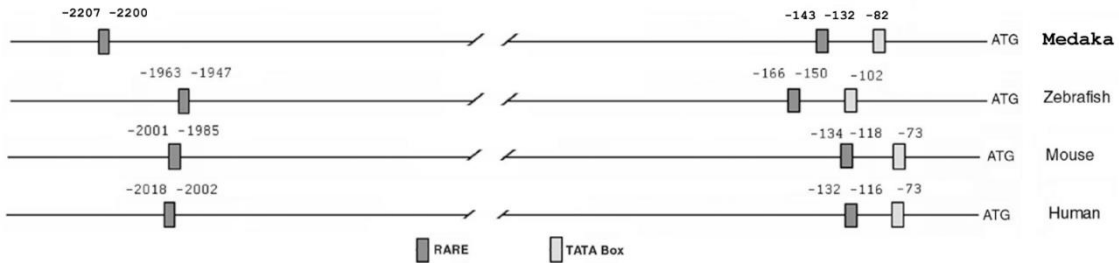


Supplementary Figure 2. Comparison between *aldh1a2* expression and responsiveness to retinoic acid in medaka embryo. (A) *in situ* hybridization for *aldh1a2* with ventral and dorsal expression domains in the retina. (B) RA responsiveness in the ventral retina. (C) *in situ* hybridization for *aldh1a2* showing expression in the first four somites. (D) GFP expression in the first four somites and the neural tube of a *tg(12xRARE:GFP)* embryo. The asterisk indicates the region where RA action is not restricted to its source of synthesis.



Supplementary Figure 3. 12XRARE transgenic signal after ATRA, DEAB and Citral. Treatment of embryos with 100 nM ATRA (C and H) starting at stage 14 induces transgene expression throughout the neural tube and neural retina by stage 23 (arrow head) compared to the DMSO-treated control (A, B, F and G). Treatment of embryos with 5 μ M DEAB (D and I) and 1 μ M Citral (E and J), both RA synthase inhibitors, at the same stage significantly reduces transgene signal in the neural tube (arrow head) compared to DMSO-treated control.

-2392 ACAGTGTGACGCTGAACCCAGATGAGGTTTGGTGGCTGTTTCTGTACCAAAGTCGATTTCACCTGTGAGATGATCAAAAACAATCTGTGCTTGACT -2293
-2292 CCCAAAATAGCATCGTGTACCCGGCTACGGCTCCCAATTGAACCTCAGACGTTCCATTGACTTTTCTTTTTTTGACGGCTTAAGCTGAACCTGGAACCA -2193
-2192 GACCTTGTCTGTATCAGTGGATATCACCAAACCTTAACTTAAATCTGAATCAAAACCCAGAAAACCTTTTACACGAGGAAATGAATGAAGCCCACTG -2093
-2092 GTCCAGGTCTGTTTTTTGGTACACCTCTGACTAGGACAGGATCCACTAAAGTCTCTAATGGAAATCAATCCGCTCTTCTCTAAGTACATAAAAAGTC -1993
-1992 CTTCCATAAGTAAATTTAAAAAAAACCTAAATTTCAAATGACTTTTATTCATAACACTGAAAGTAAAGTTCATTTAAATGCCTTTTTTCCACACCTAAGG -1893
-1892 TCACAGGGCAAAACGAATAATGAACAAAATGTTTTATCTATAAACTTAAAGATGGAGCAGATCAGGACTCCACCCTGCAGAAACAAGATAGCCATAAACAG -1793
-1792 ACGAGTTTCAGTTTTTATTTGAATTGTGAAAACAGATAGTGTTCGGAGATGTAGACAGGATCCATACCTGGGGGCAGACTGCTCTCTGTCCAATGG -1693
-1692 AAGTGAACAAGCAGAGTGGGGAGGGGGTGGAGTTATGGAGATAGCCAGACGAGATCAGAGCCGAGGCTGTGGACGGTCTTGAAGTAAAAACACGCTCT -1593
-1592 GTGACATTTGAACGGAGGTGATGCTAAAACCTGGACAGGATAAAGCGCTTCACTCTTCGTTTACTACTTGGTGACGGTGGAGGCTGGCTGCCAGTTCCAC -1493
-1492 CTACGGTCCCTCTGACCAACACCAGGATCATTTCATGCACAATCATGATGGTAGGACAGTAGCGTTAAAGGCTTGGCCAAAGGGCCCTCACTGGGTGTTACC -1393
-1392 TGAATGGTGTCTGTCTACCAATTCATTTCTTGTCTTTCATTCGCCGCCACGAAATGAACCCCTGCATGTCAGTCTTCCCTTACCAACCCAGGCTACC -1293
-1292 CAGCCGCTCATGTGTAGTGTGACATGTATAGTAGGACAGACTCTGAACACCAGAGCTGACTAAGAGAGGATGGACAGATTTGAACCTGCTGCTGAAAG -1193
-1192 CAGATTAGGCATGGATGGAGTCAAAGGCCCTCGCTCCGACCACGCAGGATGAACCCCGACGGCGAGGAGGAGGTCAGTAGTTTGGATTGAAGCAGTCAG -1093
-1092 TCCTGTGAAAGGGAGAGGAGGAAACCCCGCTTATTCAAATGATTTTCATAAAAGACATTTTCAATGGAAAATGTTGAGATCATTTGAACCCAGCAGCA -993
-992 GTTTTAGAAATGAGAGAGGAGAGAGTCTGTGAGGAGAGATGCCAAAGAGGAGGTGGGGCTTTGAGAGCAGCAGAGGGAGGGAGATCCTCTGTATGGTTCC -893
-892 TGTGTGATGAGACATGCAGTCACTTTAGTGACAAATGAGAAGAAAATAAAGGTTTAAAAAATAAAGCGTGTAAAAGCACAGCCAGCCGCTGTAACCTCAGAC -793
-792 TGCTGGCTCCCGAGTCCCGCGCTGCGCGCCACCCACATACCTGATTGAATGACGACAGCGTCAGGGATCCAAACACAACAGATCCACGCGCAAATG -693
-692 GGGATGTCGTGGTGCAGTGTGCCCCCCCCCTGTTGGGTGCAGTGGCGTGGCCCGGGGATAACCTGCGTGCCTTTCGCGCGCGCCCTTGCAT -593
-592 GCCTCATCTGACCCAAAGTTTGAACCCGGACAGAGATATCATTATAGCCCGCGCGGCTATGTGGAGGGTAAAGGGGTTCTTGGGGGCCCGTGTCCG -493
-492 GGCGTCCCGGGCCCGCGTGCCTGCAATGTTGCGTTCAGGGGCCCCGAAGAGCCTCTTACCATTAGTAAAAGCCCGCTAAGCCCGTTTGTCTG -393
-392 TGAATGACAGCCGTCGCCCTGCAGGCATTTGTGCTGTTCCCTAAGTGGCTCTTGGCGCGTTCATGAGGGTAAACAAGGCGCTCGCGCCCGCAGTCATT -293
-292 GTCTTCGGGGAGAAATTTCTGGGGACTTTCAGCGGATGTCTGCGTGCACAGGAGGTTGAGATTAAGATGCACACTTGAACAAATTTATGAGGCAA -193
-143 -132
-192 ATCAGCCAATGCAGGCCAGAGCGGGGCGAGCAACACCCCGCAATAGGCTGAACCTCTCCGGAACCTGACTCATCTGAGGGAGGTGAGGGCTGCGCGCGC -82 -93
-82 -1
-92 TCCTGCAACCTATAAAGGGTCCGCGGGGAGCGCGGGGACCCAGTCTGTGGGAGACGTTCACTGTAGCCGACGCGCCCGCACACCGCGACATG



Supplementary Figure 4. Sequence of medaka promoter region of *cyp26a1*. RA responsive elements (RARE) in the *cyp26a1* medaka promoter region. Those elements are conserved in the promoter of this gene in different species. Promoters sequences were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov>). Scheme adapted from ref.³².