

## Supplemental Material for

### **The shift from early to late types of ribosomes in zebrafish development involves changes at a subset of rRNA 2'-O-Me sites**

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**Figure S1.** Examples of validation of rRNA ribose-methylated sites by primer extension. **(A)** Primer extension analysis using high and low dNTP concentrations addressing SSU-A1031 and SSU-U1045. **(B)** Same as in A but addressing SSU-A1637. **(C)** Same as in A but addressing LSU-U4272 and LSU-U4276. All primer extension results are summarized in **Table 1** and the oligos used are listed in **Table S5**. Note that sites are numbered according to human rRNA.

**Figure S2.** Heatmap and cluster analysis, and correlation between ribose methylated sites. **(A)** Heatmap and unsupervised cluster analysis of RiboMeth-seq scores (fraction methylated) from methylated positions in SSU, 5.8S, and LSU rRNA, respectively.

**Figure S3.** 2D structure of zebrafish early-SSU rRNA. Capital and black “M” represents 2’-O-Me sites conserved between zebrafish and human, capital and blue “M” represents 2’-O-Me sites novel to zebrafish.

**Figure S4.** 2D structure of zebrafish late-SSU rRNA. Capital and black “M” represents 2’-O-Me sites conserved between zebrafish and human, capital and blue “M” represents 2’-O-Me sites novel to zebrafish.

**Figure S5.** 2D structure of zebrafish early-LSU rRNA, 5.8S, and 5S. Capital and black “M” represents 2’-O-Me sites conserved between zebrafish and human, capital and blue “M” represents 2’-O-Me sites novel to zebrafish.

**Figure S6.** 2D structure of zebrafish late-LSU rRNA, 5.8S, and 5S. Capital and black “M” represents 2’-O-Me sites conserved between zebrafish and human, capital and blue “M” represents 2’-O-Me sites novel to zebrafish.

**Figure S7.** Drawings of SNORD-rRNA interactions. G-C and A-T pairs are indicated by a line, G-U pairs by a dot, and the methylated nucleotide by an “m” above the nucleotide. The sequence of the box D or D’ upstream of the antisense element is squared.

**Figure S8.** Compensatory base pair changes. All of the interactions between the SNORDs belonging to the minimal set and ribosomal RNA (see **Fig. S7**) were searched for compensatory base pair changes in comparisons between early- and late-rRNA from zebrafish and human rRNA. Nucleotides involved in compensatory changes or establishment of additional base pairing are labeled in green. Using the early-rRNA as reference, nucleotides that differ but maintain base pairing are labeled in yellow, and deviations that disrupt base pairing are in red.

**Figure S9.** *Danio rerio* FP101887. Exons (in blue) annotated by alignment with transcript FP101887.1-204. The sequences of the SNORDs are underlined from box C to box D (both included).

**Figure S10.** Potential to form terminal stems in SNORDs encoded by the FP101887 host gene. The canonical kink-turn in box C/D snoRNAs is shown with standard symbols for non-Watson-Crick base pairs and indication of the position of the terminal stem. Sequences of the proposed base pairings for all SNORDs in the host gene are shown in their transcriptional order.

**Table S1.** Sites with an RMS-score >0.75 in at least one sample but lacking further evidence from primer extension and/or SNORD finding.

rRNA	Human	Early	Late	Primer extension
18S	G721	G769	G733	NA
18S	U1045	U1109	U1066	-
28S	G1793	G1387	G1372	-
28S	G2690	G2138	G2122	-
28S	G2692	G2140	G2124	-
28S	G4065	G3306	G3200	NA
28S	Ψ4412*	U3710	U3560	NA
28S	G4797	G4041	G3891	-
5.8S	G134	G137	G135	-

-: no primer extension signal detected, NA: not analyzed.

\*LSU:U4412 is a pseudouridine in human rRNA.

**Table S2.** List of methylated sites in zebrafish rRNA and conservation with human rRNA.

SSU (18S rRNA)							
Human	Early	Late	Conservation	Human	Early	Late	Conservation
A27	A27	A27	x	G601	G647	G611	x
A99	A99	A99	x	U627	U673	U637	x
U116	U116	U116	x	G644	G690	G654	x
U121	U121	U121	x	A668	A714	A678	x
A166	A164	A163	x	U799	U853	U815	x
U172	U170	U169	x	A1031	A1095	A1052	x
U286	U333	U296	Z	C1272	C1339	C1294	x
U287	U334	U297	Z	U1288	U1355	U1310	x
C346	C392	C356	Z	G1322	G1389	G1344	Z
U354	U400	U364	x	U1326	U1393	U1348	x
U428	U474	U438	x	G1328	G1395	G1350	x
G436	G482	G446	x	A1383	A1450	A1405	x
C462	C508	C472	x	U1442	U1507	U1461	x
A468	A514	A478	x	G1490	G1555	G1509	x
A484	A530	A494	x	A1637	A1702	A1656	Z
G509	G555	G519	x	A1678	A1743	A1697	x
A512	A558	A522	x	C1703	C1768	C1722	x
C517	C563	C527	x				
LSU (28S rRNA)							
Human	Early	Late	Conservation	Human	Early	Late	Conservation
A389	A400	A398	x	G3771	G3052	G2940	x

A391	A402	A400	x	C3787	C3068	C2956	x
G1303	G925	G913	x	ψ3797*	U3078	U2966	x
A1310	A932	A920	x	A3804	A3085	A2973	x
A1313	A935	A923	x	A3809	A3090	A2978	x
G1509	G1120	G1109	x	C3820	C3101	C2989	x
A1511	A1122	A1111	x	C3848	C3129	C3017	x
A1521	A1132	A1121	x	C3866	C3147	C3035	x
G1612	G1223	G1212	x	G3878	G3159	G3047	x
A1858	A1454	A1437	x	U3904	U3185	U3073	x
C2338	C1785	C1770	x	C3916	C3197	C3085	Z
A2350	A1797	A1782	x	G3923	G3204	G3092	x
G2351	G1798	G1783	x	C4032	C3273	C3167	x
C2352	C1799	C1784	x	G4166	G3464	G3314	x
U2402	U1849	U1834	x	U4197	U3495	U3345	x
G2411	G1858	G1843	x	G4198	G3496	G3346	x
A2774	A2222	A2206	x	U4272	U3570	U3420	Z
C2791	C2239	C2223	x	U4276	U3574	U3424	x
A2802	A2250	A2234	x	G4340	G3638	G3488	x
G2817	G2265	G2249	Z	G4362	G3660	G3510	x
U2824	G2272	G2256	x	C4426	C3724	C3574	x
C2848	C2297	C2280	x	G4464	G3762	G3612	x
G2863	G2312	G2295	x	U4468	U3766	U3616	x
G3606	G2887	G2775	x	G4469	G3767	G3617	x
C3680	C2961	C2849	x	A4493	A3791	A3641	x
A3697	A2978	A2866	x	C4506	C3804	C3654	x
A3703	A2984	A2872	x	A4560	A3860	A3709	x
G3723	G3004	G2892	x	U4590	U3890	U3739	x
A3739	A3020	A2908	x	G4593	G3893	G3742	x
A3764	A3045	A2933	x	C4983	C4186	C4022	Z

### 5.8S rRNA

Human	Early	Late	Conservation
U8	U8	U8	Z
U14	U14	U14	x
G75	G75	G75	x

x: Methylated sites conserved between zebrafish and human rRNA.

Z: novel methylation identified in zebrafish not found in human.

\*LSU:U3797 is a methylated pseudouridine (Ψm) in human rRNA.

**Table S3.** Novel zebrafish methylated sites not found in human rRNA.

rRNA	Human	Early	Late	rRNA	Human	Early	Late
18S	U286	U333	U296	28S	G2817	G2265	G2249
18S	U287	U334	U297	28S	C3916	C3197	C3085
18S	C346	C392	C356	28S	U4272	U3570	U3420
18S	G1322	G1389	G1344	28S	C4983	C4186	C4022
18S	A1637	A1702	A1656	5.8S	U8	U8	U8

**Table S4.** Assigned SNORDs.

<b>SSU (18S rRNA)</b>					
Nucleotide	SNORD	Nucleotide	SNORD	Nucleotide	SNORD
A27	SNORD27	C462	SNORD14	C1272	SNORD66
A99	SNORD57	A468	SNORD83	U1288	SNORD110
U116	SNORD42	A484	SNORD16	G1322	NA
U121	SNORD4	G509	SNORD11	U1326	SNORD33
A166	SNORD44	A512	SNORD70	G1328	SNORD32
U172	SNORD45	C517	SNORD56	A1383	SNORD30
U286	NET1	G601	SNORD103	U1442	SNORD61
U287	NET1 (+6 mod.)*	U627	SNORD65	G1490	SNORD25
C346	SNORD200	G644	SNORD54	A1637	NA
U354	SNORD90	A668	SNORD36	A1678	SNORD82
U428	SNORD68	U799	SNORD19	C1703	SNORD43
G436	SNORD100	A1031	SNORD59A/B		
<b>LSU (28S rRNA)</b>					
Nucleotide	SNORD	Nucleotide	SNORD	Nucleotide	SNORD
A389	SNORD26	U2824	SNORD34	C3916	SNORD202
A391	SNORD81	C2848	SNORD50A	G3923	SNORD111
G1303	SNORD21	G2863	SNORD50B	C4032	SNORD75
A1310	SNORD126	G3606	SNORD201	G4166	SNORD31
A1313	SNORD18	C3680	SNORD88	U4197	SNORD58
G1509	SNORD2	A3697	SNORD37	G4198	SNORD58 (+6 mod.)*
A1511	SNORD51	A3703	SNORD36	U4272	SNORD203
A1521	SNORD77	G3723	SNORD87	U4276	SNORD41
G1612	SNORD80	A3739	SNORD46	G4340	SNORD60
A1858	SNORD38	A3764	SNORD15	G4362	SNORD1
C2338	SNORD24	G3771	SNORD15	C4426	SNORD49
A2350	SNORD76	C3787	SNORD10	G4464	SNORD69
G2351	SNORD76 (+6 mod.)*	ψ3797**	SNORD17	U4468	Spb1 (yeast)***
C2352	SNORD24	A3804	SNORD30	G4469	Spb1 (yeast)***

U2402	SNORD143	A3809	SNORD79	A4493	SNORD29
G2411	SNORD6	C3820	SNORD74	C4506	SNORD35
A2774	SNORD99	C3848	SNORD53A/B	A4560	SNORD119
C2791	SNORD55	C3866	SNORD47	U4590	SNORD72
A2802	SNORD95	G3878	SNORD12	G4593	SNORD78
G2817	NA	U3904	SNORD52	C4983	NET3

### 5.8S rRNA

Nucleotide	SNORD	Nucleotide	SNORD	Nucleotide	SNORD
U8	SNORD204	U14	SNORD71	G75	SNORD96

mod.: modification, NA: No assigned SNORD.

\* Assumed to be +6 modifications.

\*\* LSU:U3797 is a methylated pseudouridine ( $\Psi$ m) in human rRNA.

\*\*\* Assumed to be introduced by Spb1-homolog.

**Table S5.** List of DNA oligos.

Primer extension analysis				
Name	Sequence	Description	Pos. 3'end (early)	Pos. 3'end (late)
C1594	GCAGACATTCGAATGAATCGT	px 1.1	-	346 (SSU)
C1595	ATCGGCCCGAGGTTA	px 1.2	347 (SSU)	310 (SSU)
C1596	ACCATGGTAGGCGCCTAA	px 2.1	-	389 (SSU)
C1597	TACCGGACACCCAGCTAA	px 3.1	-	780 (SSU)
C1598	CATCGTTTACGGTCGGAA	px 4.1	1136 (SSU)	1093 (SSU)
C1599	CAAATCGCTCCACCACTAA	px 5.1	-	1364 (SSU)
C1600	GACTTAATCAACGCAAGCTTA	px 6.1	-	1681 (SSU)
C1601	ACTGGGCGTCTCGCATT	px 7.1	-	1403 (LSU)
C1602	GCCAGTTCTGCTTACCAA	px 7.2	-	1428 (LSU)
C1603	AGGGCCGACGAGAGCTCA	px 8.1	2158 (LSU)	2142 (LSU)
C1604	TTCTCCCCGGATTTTCAA	px 8.2	2175 (LSU)	2159 (LSU)
C1605	CGAAGTTACGGATCTGACTT	px 9.1	2293 (LSU)	2276 (LSU)
C1606	ACTTGCCGACTTCCCTTA	px 9.2	2279 (LSU)	2262 (LSU)
C1607	GGGCCTCCCACTTATTCT	px 10.1	3220 (LSU)	3108 (LSU)
C1608	CCCAGGGCCTCCCACTT	px 10.2	-	3113 (LSU)
C1609	GCTTGACGCCAGAAGCA	px 11.1	3343 (LSU)	3228 (LSU)
C1610	CGTGAGGCCCGCTTTCA	px 12.1	-	3439 (LSU)
C1611	AAGCCAGAAGGATCGTGA	px 12.2	-	3452 (LSU)
C1612	GATCGTGAGGCCCGCTT	px 12.3	3529 (LSU)	3442 (LSU)
C1613	TCCCGTCACGGCTGGCTCT	px 13.1	-	3915 (LSU)
C1614	CCGGTTCCTGTACGAAT	px 13.3	4079 (LSU)	-
C1615	CTCTGCCACGCACGAAA	px 14.1	-	4039 (LSU)

C1616	CAGCGATGGAGCTGCTCTG	px 14.2	-	4051 (LSU)
C1617	GCAGCTAGCTGCGTTCTT	px 15.1	40 (5.8S)	40 (5.8S)
C1618	GGCGACGCTCAGACA	px 16.1	-	143 (5.8S)
C1666	AATCGAGAAAGAGCTATCAAT	px 19.1	-	1317 (SSU)
C1623	AGATCCAAAACCCATCCGGGTG	seq-temp Fo	-	248 (SSU)
C1624	GTAATTCCAGCTCCAATAGCG	seq-temp Fo	692 (SSU)	659 (SSU)
C1625	ATGGAATAGGACTCCGGTTC	seq-temp Fo	-	904 (SSU)
C1626	ATGGGCGGATCAACGTGTGC	seq-temp Fo	1618 (SSU)	1572 (SSU)
C1627	TGTGTACAAAGGGCAGGGAC	seq-temp Re	1745 (SSU)	1699 (SSU)
C1628	ACGTGCAAATCGGTCGTCGAC	seq-temp Fo	1212 (LSU)	1201 (LSU)
C1629	GGTCTGATGAGCGTCGGCATC	seq-temp Re	1499 (LSU)	1482 (LSU)
C1630	GCAGATCTTGGTGGTAGTAGC	seq-temp Fo	1799 (LSU)	1784 (LSU)
C1631	CCGAAGTTACGGATCTGACTTGC	seq-temp Re	2291 (LSU)	2274 (LSU)
C1632	GGATGAACGAGATCCCCTGTC	seq-temp Fo	3101(LSU)	2989 (LSU)
C1633	AGCCAGTTATCCCTGTGGTAAC	seq-temp Re	3650 (LSU)	3500 (LSU)
C1634	GGTGCGAAGCTACCATCTGTG	seq-temp Fo	-	3797 (LSU)
C1635	CAACTCTTAGCGGTGGATCACTC	seq-temp Fo	23 (5.8S)	23 (5.8S)
C1636	CGACGCTCAGACAGGCGT	seq-temp Re	-	138 (5.8S)

Pos: position of the 3' end of the oligo in early and late rRNA, seq-temp: oligo used for PCR to generate sequencing ladder.