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An alternative spliceosome defined by distinct snRNAs in early zebrafish embryogenesis

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Alternative snRNAs in early zebrafish embryogenesis

snRNA, spliceosome, dual translation machinery, embryogenesis

1   ABSTRACT

2   Splicing removes intronic RNA sequences are removed from pre-mRNA molecules and

3   enables, by alternative splicing, the generation of multiple unique RNA molecules from a

4   single gene. As such, splicing is an essential part of the whole translation system of a cell.

5   The spliceosome is a ribonucleoprotein complex in which five small nuclear RNAs (snRNAs)

6   are involved; U1, U2, U4, U5, and U6. For each of these snRNAs there are variant gene

7   copies present in a genome. Furthermore, in many eukaryotic species there is an

8   alternative, minor spliceosome that can splice a small number of specific introns. As we

9   previously discovered an embryogenesis-specific ribosomal system in zebrafish early

10   embryogenesis based on variant rRNA and snoRNA expression, we hypothesized that there

11   may also be an embryogenesis-specific spliceosome. An inventory of zebrafish snRNA genes

12   revealed clustered and dispersed loci for all but U2 major snRNAs. For each minor

13   spliceosome snRNA, just one gene locus was found. Since complete snRNA molecules are

14   hard to sequence, we employed a combined PCR-sequencing approach to measure the

15   individual snRNA-variant presence. Analysis of egg and male-adult samples revealed

16   embryogenesis-specific and somatic-specific variants for each major snRNA. These variants

17   have substantial sequence differences, yet none in their mRNA binding sites. Given that

18   many of the sequence differences are found in loop structures indicate possible alternative

19   protein binding. Altogether, with this study we established that the spliceosome is also an

20   element of the embryogenesis-specific translation system in zebrafish.

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28 INTRODUCTION

29 Alternative splicing is fundamental for gene regulation and the generation of different

30 transcripts and/or proteins from an individual gene in eukaryotes (1). Splicing is executed by

31 the spliceosome and removes intronic sequences from pre-mRNA during the maturation

32 process in which the exonic sequences eventually form the mRNA (2,3).The spliceosome is a

33 molecular complex formed by hundreds of proteins and five essential small-nuclear RNAs

34 (snRNAs) that are typically located in the nucleus. The size of these small RNA molecules

35 ranges from 118 nucleotides (nt) to 191 nt. As they are uracil rich, they are called U1, U2,

36 U4, U5 and U6 snRNAs. Next to this major spliceosome, a minor (or U12 dependent)

37 spliceosome exists in many eukaryotic species, which is involved in the splicing of a relative

38 small number of specific introns (4). The snRNAs involved in the minor spliceosome are:

39 U11, U12, U4atac, and U6atac, completed by the U5 from the major spliceosome (4).

40 As splicing is at the core of the cellular translation system, the sequences of the involved

41 snRNA are highly conserved across species. At the same time, many non-canonical variants

42 and gene copies of the major snRNA genes are present within the same organisms (5–9).

43 This raises the question why these variants exist and what role they might play in

44 translation. Although expression of these variants has been extensively studied, there is still

45 not a clear understanding for the existence of these snRNA variants (10).

46 A indication to the function of variant snRNAs may lie in the fact that several studies have

47 observed differentially expression of variants during development. For instance in

48 Drosophila several variants are expressed during early embryogenesis, but eventually one

49 variant gradually dominates expression (11,12). Similar expression patterns for snRNA

50 variants were observed in *Xenopus* (13–15), mouse (12,16), sea urchin (17,18), and flatworm

51 (19). Comparable findings have been reported for: snRNA U2 in silk moth (20) and

52 *Dictyostelium dicoideum* (21); snRNA U4 in chicken (22); and snRNA U5 in *Drosophila* (5) and

53 in human (6). snRNAs also display a tissue-preferred expression, which implicate them in

54 different disease pathologies such as several neurological diseases (7,10,23,24).

55 The fact that there are snRNAs which variants are differentially expressed during

56 embryogenesis relates to our previous findings where we discovered distinct maternal-types

57 of rRNAs and snoRNAs specifically expressed during early zebrafish embryogenesis (25).

58 These maternal-type RNAs seem to be part of a distinct early embryogenesis-specific

59   translation machinery, which is gradually replaced during embryogenesis by a somatic type.

60   Combining the snRNA and our rRNA plus snoRNA findings, lead us to hypothesize that there

61   might also be distinct maternal-type snRNAs contributing to the embryogenesis-specific

62   translation machinery.

63   As in our hands, snRNAs cannot consistently be sequenced by standard next-generation

64   sequencing protocols, probably due to strong secondary structures and ample RNA

65   modifications, we employed a wet-lab approach combining RT-PCR, for amplification, and

66   DNA sequencing, for identification, of snRNAs. Using this approach on egg and mature adult-

67   male samples, we were able to show that for each snRNA, similar to rRNA and snoRNA,

68   there are maternal-type snRNAs that are uniquely present in zebrafish eggs. This means that

69   snRNAs are a part of the zebrafish unique embryogenesis-specific translation machinery.

70   RESULTS AND DISCUSSION

71   Cataloguing the zebrafish snRNAs

72   In earlier studies we discovered cellular components related to the transcription machinery

73   that have distinct embryogenesis expression or somatic expression during zebrafish

74   development. We reported on 5S (26), 5.8S, 18S, and 28S (27), plus small-derived

75   components from those molecules (28), as well as snoRNAs (25). As it is clear that in

76   zebrafish oogenesis and early embryogenesis a different transcription machinery is

77   employed, it is obvious to investigate other components of this system. Hence, we here

78   focused on the small nuclear RNAs (snRNAs) of the spliceosome to determine whether their

79   variants also display distinct expression profiles. We started by making an inventory of all

80   snRNA elements present in the zebrafish genome based on the annotated snRNA sequences

81   from the Ensembl database (29). In total, 541 snRNA loci were retrieved for the major

82   spliceosome and seven snRNA loci for the minor spliceosome from the database. Given the

83   many snRNA loci for the major spliceosome, we compared them by sequence alignment per

84   snRNA (Supplemental Figure SF1). Several snRNA sequences appeared aberrant and it

85   turned out that these sequences partly existed of retrotransposon sequences (30), therefore

86   they were excluded (Supplemental Table ST1). To complete our reference set of snRNAs, the

87   database-derived snRNA sequences were used to explore the zebrafish genome for yet

88 unannotated major and minor spliceosome snRNA loci. This resulted in a total of 958 snRNA

89 loci (Table 1, Supplemental Table ST1 and File SFile1).

90 The alignment of the sequences within each major spliceosome snRNA showed two clusters

91 for each snRNA (Supplemental Files SF1). The distribution of these two snRNA clusters

92 coincides with a clear genomic organizational preference: either in condense genomic

93 repeats (Table 1, blue), or scattered throughout the genome (Table 1, red). Obviously, the

94 discovery of two sequence clusters for each major spliceosome snRNA is in line with the

95 possible existence of maternal-type and somatic-type snRNA spliceosomes.

96 In contrast; for the minor spliceosome, U12 snRNA is present only once in the genome,

97 which means that for U12 snRNA no maternal-type variant can exist. For all three other

98 minor spliceosome-specific snRNAs, just two loci appeared to be present in the genome

99 (Supplement Table ST1). Because the observed sequences within each of these minor

100 spliceosome snRNAs are so different, we questioned whether they are legitimate snRNA

101 loci. By analyzing the expression of all these snRNA loci, it could be determined that for each

102 minor spliceosome snRNAs, only one locus is expressed (Table 1, grey, Supplemental Table

103 2) This precludes the existence of an alternative, completely embryogenesis-specific minor

104 spliceosome and we therefore excluded these minor spliceosome snRNAs from the

105 subsequent analyses.

106

Table 1: Genomic distribution of maternal and somatic zebrafish snRNA genes

	total #	Chromosome
		2	3	4	5	6	7	8	9	11	13	17	19	20	21	25
major snRNA																
U1	259			248			4	2	3					2		
U2	7	2				1	2				2					
U4	26			2								23				1
U5	139				132				1					6		
U6	520		12	503		1			1	1				1	1	
minor snRNA																
U11	1												1			
U12	1		1													
U4atc	1									1						
U6atc	1														1	

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Embryogenesis-specific snRNA variants

110 After cataloguing all snRNAs in the zebrafish genome, we investigated whether

111 embryogenesis-specific snRNAs exist by examining their expression in eggs and somatic

5

112 tissue. However, it became quickly evident that (complete) snRNA molecules, similarly to

113 tRNA, 5S, and snoRNA molecules, cannot readily be sequenced by standard smallRNA-seq

114 procedures, probably due to their robust secondary configurations, as well as possible 3'

115 modifications. Only small numbers of partial snRNA sequences were observed, which did

116 show a hint of differential snRNA expression between egg and adult tissue. To tackle this

117 technical problem, we performed a RT-PCR-qSeq analysis effectively circumventing the

118 snRNA 3' issues. For this, we selected the sequence most prominently present in the

119 genome for each cluster of every snRNAs of the major spliceosome (Figure 1A). The

120 differences between these pairs of snRNA sequences ranges from 11 nucleotides (U1 and

121 U6) to 48 nucleotides (U5) (Figure 1A, Supplemental Figure SFig1). We designed

122 (degenerated) RT-PCR-primers (Figure 1A) that amplified virtual all snRNAs, after which the

123 PCR products were sequenced and identified by mapping to the snRNA sequences. Using

124 this procedure at least 0.5 M reads were obtained for each snRNA (Supplemental Table

125 ST2). These results revealed that for each snRNA one variant accounts for almost all snRNA

126 in the egg samples, whereas the other variant makes up the snRNAs in adult zebrafish

127 (Figure 1B). Similar to rRNA and snoRNA, the snRNA variant present in egg is called

128 maternal-type versus the somatic-type variant in adult tissue (Figure 1A).

129 To check the RT-PCR-qSeq results we developed a qRT-PCR analyses for snRNA U1 and

130 snRNA U5. This qRT-PCR analysis confirmed the exclusive presence of embryogenesis-

131 specific snRNA variants in egg samples (Supplemental Figure SFig2).

132 **Figure 1. Maternal-type and somatic-type snRNA sequences and expression**

133 **A**: Sequence comparison of selected maternal-type (M) and somatic-type (S) snRNA variants;

134 identical nucleotides are indicated as dots, while gaps as dashes (For sequence alignment of all

135 snRNAs cf. Supplemental Figure SF2). The RT-PCR primers are indicated with half arrows: maternal-

136 type specific (blue); somatic-type specific (red); and non-distinctive (green).

137 **B**: Relative expression of maternal-type (blue) and somatic-type (red) variants for each snRNA

138 indicated by comparative percentage calculated using the RT-PCR-qSeq approach on RNA from egg

139 and male tail tissue (MT).

140

141 Differences between maternal- and somatic-types snRNAs

142 In order to assign any functional relevance to the embryogenesis-specific snRNAs,

143 nucleotide difference as compared to the somatic-type snRNAs were investigated (Figure

144 1A). One of the distinctions between the major and minor spliceosome snRNAs lies in their

145 nucleotide sequences that bind to the mRNA, thus allowing each system to splice distinct

146 introns. However, even though there are many sequence differences between the maternal-

147 type and the somatic-type snRNAs, none of them involve the mRNA binding sites in these

148 snRNAs (Figure 2 and Supplement File SF3). It turned out that the snRNA sequence

149 differences are often located in specific parts of the secondary structure (Figure 2). For

150 instance, for U1, all but one differences are located in one stem-loop and in U2 they are

151 confined to one side of the structure (Figure 2). In general, many of the differences are

152 found in the loops, which are thought to be specific binding locations for spliceosomal

153 proteins. Hence, this would indicate that the embryogenesis spliceosome, besides specific

154 snRNAs also may comprise (embryogenesis-)specific proteins.

155 Despite many apparently co-evolved nucleotide pairs in stems of the snRNAs (Figure 2),

156 there seems to be only one co-evolved nucleotide pair in the interaction site between U4

157 and U6. However, as this nucleotide is right in the middle of the largest interaction site, it

158 might actually prevent the binding of somatic-type U4 to maternal-type U6 and vice versa.

159

160 **Figure 2. Sequence differences between maternal-type and somatic-type snRNAs**

161 The secondary structure is presented for each maternal-type and somatic-type zebrafish snRNA.

162 These structures are an adaptation from Figure 4 in reference (4) and also indicate the specific

163 binding sites between U4 and U6. The arrows indicate the base pair that coevolved within one of the

164 U4 – U6 interaction sites. The circles indicate nucleotides that are different between the maternal-

165 type (blue) and somatic-type (red) sequences. The sites interacting with the pre-mRNA are

166 underlined.

167

168 CONCLUDING REMARKS

169 In this study we reveal the existence of an embryogenesis-specific major spliceosome in

170 zebrafish, consisting of at least a distinct set of U1, U2, U4, U5 and U6 snRNAs. We do not

171 know if this embryogenesis-specific spliceosome also contains embryogenesis-specific

172 proteins, yet the position of the distinguishing nucleotides in the loops of the maternal-type

173 snRNA structure would suggest this. However, a quick scan of some of the major

174 spliceosomal protein genes revealed that they are present with just one copy in the

7

175   zebrafish genome, which effectively rules out any embryogenesis-specific spliceosomal

176   protein gene variants. Yet, the genes for these proteins do contain introns, which have in

177   general an important function with respect to production of alternative transcripts plus

178   alternative proteins. This leads to the intriguing question, whether the maternal-type

179   snRNAs will produce embryogenesis-specific alternative-spliced transcripts and thus

180   embryogenesis-specific spliceosome proteins for the embryogenesis-specific variant of the

181   major spliceosome.

182   With respect to the minor spliceosome, which acts on different splicing sites; we did not find

183   any maternal-type snRNA variants for U11, U12, U4atc, and U6atc. This however does not

184   lead to the conclusion that no embryogenesis-specific minor spliceosome exists. On the

185   contrary, the minor spliceosome is always completed by the U5 snRNA from the major

186   spliceosome (4) and because in eggs virtually only the maternal-type U5 snRNA is present,

187   this U5 variant is used in combination with the other four ubiquitously-expressed minor

188   spliceosome snRNAs. Even though only one snRNA in this splicing system is different, we

189   would argue that this makes the whole minor spliceosome in eggs embryogenesis-specific.

190   This might also explain the observation that the maternal-type U5 snRNA sequence differs a

191   noteworthy 41% compared to the somatic-type, whereas in all other snRNAs this difference

192   is just 7% to 20%. Yet, the mRNA binding loop that holds the exons together (32) and the

193   associated stem are identical between maternal-type, somatic-type and human U5 snRNA.

194   Besides this core the zebrafish U5 snRNA variants are almost completely different (53%).

195   The functional implications remain elusive for now.

196   Although the differences between the variants of each snRNA are quite substantial, their

197   secondary structure appears to be relatively unaltered. This is also due to many co-evolved

198   nucleotides in the stem sequences. Similar to what we observed previously for the 45S

199   rRNAs, the sequence homology between the somatic-type and maternal-type snRNA

200   variants is often lower than snRNAs in distantly-related species. For instance, apart from the

201   U1 snRNA, the sequence homology between maternal-type and somatic-type zebrafish

202   snRNA variants is lower than between the somatic-type zebrafish variant and the human

203   snRNA sequences (Supplemental Figure SFig1). The considerable sequence difference of the

204   snRNA variants within the zebrafish hints on an intriguing need for two very different

205   spliceosomal systems in early embryogenesis and adult zebrafish.

206   The genomic distribution of the maternal-type and somatic-type snRNA variants is also

207   similar to that of the small snoRNAs and 5S rRNAs in that U1, U5 and U6 have a relative high

208   number of maternal-type snRNA genes, whereas there are only a few somatic-type genes.

209   This correlates with the fact that the U1, U5 and U6 are the snRNA that directly interact with

210   the mRNA. However, it is still somewhat surprising that the maternal-type U2 variant is

211   present only in two loci. Also similar to 5S rRNA, the majority of multiple U1, U5 and U6

212   snRNA genes are organized in a strictly repeated manner, but with several interruptions

213   caused by retrotransposons (26). It was also clear that some snRNA genes were only

214   partially present in the genome (Supplemental Table ST1). Together this would be in line

215   with the reported presence of retrotransposons in snRNA gene repeats (33,34). A quick scan

216   of these snRNA interrupting sequences, revealed the presence of retrotransposons with and

217   without intact open reading frames. Alike 5S loci, we expect that retrotransposons might

218   play a role in maintaining the needed number of gene copies in the snRNA repeats.

219   The strict differential expression of maternal-type and somatic-type snRNAs obviously is

220   regulated via the promotor and auxiliary sequences related to the snRNA genes and we

221   expect that an extensive analysis of all maternal-type and somatic-type snRNA promoter

222   regions will eventually discover the relevant differences. This holds also for mechanisms

223   that are involved in snRNA processing and degradation. For instance, a superficial

224   comparison suggests that the core sequences of the 3' box, which is involved in the post

225   transcriptional processing of snRNAs (35), are different between the maternal-type and

226   somatic-type snRNA variants (results not shown).

227   In many other species different snRNA variants have been found, often with differential expression

228   during embryogenesis, such as in *Xenopus* (13), Human (36), mouse (37), sea urchin (17), *Drosophila*

229   (12). The observed zebrafish snRNA variant system is unique in that the maternal-type snRNA is

230   exclusively expressed in oocytes, eggs, and early embryogenesis. All other reports just mention up to

231   40% higher expression of some variants. This implies that by means of maternal-type snRNAs,

232   together with the maternal-type rRNAs and maternal-type snoRNAs we previously reported,

233   zebrafish employ a distinct translation system specifically for early embryogenesis

234

235   MATERIAL AND METHODS

236   Biological materials

237   Adult zebrafish (strain ABTL) were handled in compliance with local animal welfare

238   regulations and maintained according to standard protocols (http://zfin.org). The breeding

239   of adult fish was approved by the local animal welfare committee (DEC) of the University of

240   Leiden, the Netherlands. All protocols adhered to the international guidelines specified by

241   the EU Animal Protection Directive 86/609/EEC.

242   For this study samples were used of two pools of unfertilized eggs (oocyte clutches) and two

243   whole male adult zebrafish. The harvesting of the biological materials and RNA-isolation

244   have been described previously (26,27).

245   Source data

246   In this study we use the zebrafish genome version GRCz11 and next-generation sequencing

247   data previously generated by our group (26) and available through the BioProject database

248   with accession number PRJNA347637.

249   qRT-PCR analysis

250   For snRNA U1 and U5, forward and reverse PCR primers were used from the RT-PCR-qSeq

251   analysis, and quantitative real-time PCR (qRT-PCR) probes were designed (Supplemental

252   Table ST4). Reverse transcription was done in two independent reactions for zebrafish

253   clutch (= egg pool) and adult male tail total RNA. SuperScript IV Reverse Transcriptase

254   (Thermo Fisher Scientific) was used according to the manufacturer's instructions. Separate

255   qRT-PCR analyses were performed on 10-fold dilutions of the cDNAs with the snRNA U1 and

256   U5 primer/probe combinations using a QuantStudio 3 Real-Time PCR System (Thermo Fisher

257   Scientific).

258   RT-PCR-qSeq analysis

259   Forward and reverse PCR primers were designed for maternal-type and somatic-type snRNA

260   variants, in such a way that: 1) as much as possible of the 5'-end of the full-length variants is

261   included in the final amplicon, and 2) generic primers will bind to the maternal-type, as well

262   as the somatic-type variants (Supplemental Table ST4 and Figure 1A). To avoid positive

263   results due to genomic DNA background, small RNA-enriched total RNA was treated twice

264   with 5 µl of RNase-free DNase (Qiagen) for 45 minutes at 37°C. Next, cDNA was prepared

10

265   from 50 ng of RNA as described in (26). For each sample, reverse transcription was primed

266   using a mixture of all reverse PCR primers. Controls without reverse transcriptase were used

267   to exclude genomic DNA contamination of the RNA in the downstream PCR. Subsequently,

268   standard PCR reactions were performed on 1 ul of cDNA using Q5 High-Fidelity DNA

269   Polymerase (New England Biolabs) and each of the variant PCR primer pairs independently.

270   The resulting amplicons were purified using the QIAquick PCR Purification Kit (Qiagen)

271   according to the manufacturer's instructions, with the exception that a total of seven

272   volumes of solution PB was added to allow for more efficient binding of fragments <100 bp.

273   Next, the PCR products were phosphorylated using T4 PNK (New England Biolabs) and again

274   purified as described above. Afterwards, the size of the PCR product was verified on a 2200

275   TapeStation System (Agilent). From 44 ng purified phosphorylated PCR product, barcoded

276   sequencing libraries were prepared using a modified version of the Ion Xpress Plus Fragment

277   Library Kit (Thermo Fisher Scientific) as described previously (26). Massive-parallel

278   sequencing was performed on an Ion Proton System (Thermo Fisher Scientific) using an Ion

279   PI Chip Kit v3.

280   Bioinformatics analyses

281   *Known snRNA sequences.* The initial set of snRNA sequences of *D. rerio* (GRCz11) were

282   downloaded from Ensemble 95 (29) in October 2018 using Biomart by selecting snRNA as

283   *Gene type* (See the RF annotated tabs in Supplementary Table1).

284   *Discarding "contaminated" snRNA sequences.* For each of the five snRNAs a multiple

285   alignment of the downloaded sequences was made. This was done using CLC Genomic

286   Workbench with default settings (gap open cost 20, gap extension 20 end gap cost free, very

287   accurate). Via visual inspection of the alignments (Supplementary Figure SF1) the snRNA

288   sequences that contained obvious contaminating, non-snRNA sequences, such as

289   retrotransposon sequences, were discarded or truncated.

290   *Discovery of new snRNA sequences and loci.* The known snRNA sequences were then aligned

291   with the zebrafish genome using BLASTN and all filters and masking unselected. The hits

292   that were at least 95% of the query length were selected. Names were then assigned to the

293   unique sequences.

11

294 *Mapping of the smallRNA-seq reads.* The reads from the source data were mapped to the

295 major and minor snRNA sequences using default settings of Bowtie-2 (31).

296 *Analysis of the RT-PCR-qSeq.* To count the number of maternal-type and somatic-type reads

297 in egg and adult male zebrafish, an exact string search of the maternal-type and somatic-

298 type snRNA sequences was performed for each snRNA. Data normalization was done using

299 the total count of mapped smallRNA-seq reads per sample.

300

301 .ACKNOWLEDGMENTS

304

305 SUPPLEMENTAL FILES

306 SF1: Zebrafish snRNA sequence alignments

307 SF2: Alignment of snRNA sequences to selected maternal-type and somatic-type variant

308 sequences

309 SUPPLEMENTAL TABLES

310 ST1.xlsx: Genome distribution of zebrafish snRNAs

311 ST2.xlsx: Minor spliceosome snRNA read counts

312 ST3.xlsx: Read counts from the RT-PCR-qSeq experiment

313 ST4.xlsx: Primer sequences

314 SUPPLEMENTAL FIGURES

315 Sfig1.pdf: Sequence alignments of human snRNAs with maternal-type and somatic-type

316 zebrafish snRNA

317 Sfig2.pdf:qRT-PCR results for snRNA U1 and snRNA U5

318 REFERENCES

319 1. Chen M, Manley JL. Mechanisms of alternative splicing regulation: Insights from
320   molecular and genomics approaches. Nat Rev Mol Cell Biol [Internet].
321   2009;10(11):741–54. Available from: http://dx.doi.org/10.1038/nrm2777

322 2. Walter Gilbert. Why genes in pieces? Nature. 1978;271(9):501.

323 3. Shi Y. Mechanistic insights into precursor messenger RNA splicing by the spliceosome.
324   Nat Rev Mol Cell Biol [Internet]. 2017;18(11):655–70. Available from:
325   http://dx.doi.org/10.1038/nrm.2017.86

326 4. Turunen JJ, Niemelä EH, Verma B, Frilander MJ. The significant other: Splicing by the
327   minor spliceosome. Wiley Interdiscip Rev RNA. 2013;4(1):61–76.

328 5. Chen L, Lullo DJ, Ma E, Celniker SE, Rio DC, Doudna JA. Identification and analysis of
329   U5 snRNA variants in Drosophila. RNA. 2005;11(10):1473–7.

330 6. Sontheimer EJ, Steitz JA. Three novel functional variants of human U5 small nuclear
331   RNA. Mol Cell Biol. 2015;12(2):734–46.

332 7. O'Reilly D, Dienstbier M, Cowley SA, Vazquez P, Drozdz M, Taylor S, et al.
333   Differentially expressed, variant U1 snRNAs regulate gene expression in human cells.
334   Genome Res. 2013;23(2):281–91.

335 8. Boonanuntanasarn S, Panyim S, Yoshizaki G. Characterization and organization of the
336   U6 snRNA gene in zebrafish and usage of their promoters to express short hairpin
337   RNA. Mar Genomics [Internet]. 2008;1(3–4):115–21. Available from:
338   http://dx.doi.org/10.1016/j.margen.2008.10.001

339 9. Domitrovich AM, Kunkel GR. Multiple, dispersed human U6 small nuclear RNA genes
340   with varied transcriptional efficiencies. Nucleic Acids Res. 2003;31(9):2344–52.

341 10. Vazquez-Arango P, O'Reilly D. Variant snRNPs: New players within the spliceosome
342   system. RNA Biol [Internet]. 2018;15(1):17–25. Available from:
343   https://doi.org/10.1080/15476286.2017.1373238

344 11. Lo PCH, Mount SM. Drosophila melanogaster genes for U1 snRNA variants and their
345   expression during development. Nucleic Acids Res. 1990;18(23):6971–9.

346 12. Lu Z, Matera AG. Developmental Analysis of Spliceosomal snRNA Isoform Expression.
347   G3&#58; Genes|Genomes|Genetics [Internet]. 2015;5(1):103–10. Available
348   from: http://g3journal.org/lookup/doi/10.1534/g3.114.015735

349 13. Forbes DJ, Kirschner MW, Caput D, Dahlberg JE, Lund E. Differential expression of
350   multiple U1 small nuclear RNAs in oocytes and embryos of Xenopus laevis. Cell.
351   1984;38(3):681–9.

352 14. Lund E, Bostock CJ, Dahlberg JE. The transcription of Xenopus laevis embryonic U1
353   snRNA genes changes when oocytes mature into eggs. Genes Dev. 1987;1(1):47–56.

354 15. Lund E, Dahlberg JE. Differential accumulation of U1 and U4 small nuclear RNAs
355   during Xenopus development. Genes Dev. 1987;1(1):39–46.

356 16. Cheng Y, Lund E, Kahan BW, Dahlberg JE. Control of mouse U1 snRNA gene expression
357   during in vitro differentiation of mouse embryonic stem cells. Nucleic Acids Res.
358   1997;25(11):2197–204.

359 17. Nash MA, Sakallah S, Santiago C, Yu J-C, Marzluff F. Switch in Sea Urchin Ul RNA. Dev
360   Biol. 1989;296(134):289–96.

361 18. Stefanovic B, Li JM, Sakallah S, Marzluff WF. Isolation and characterization of
362   developmentally regulated sea urchin U2 snRNA genes. Dev Biol. 1991;148(1):284–
363   94.

364 19. Schmidt D, Reuter H, Hüttner K, Ruhe L, Rabert F, Seebeck F, et al. The Integrator
365   complex regulates differential snRNA processing and fate of adult stem cells in the
366   highly regenerative planarian Schmidtea mediterranea. PLoS Genet.
367   2018;14(12):e1007828.

368 20. Sierra-Montes JM, Freund A V., Ruiz LM, Szmulewicz MN, Rowold DJ, Herrera RJ.
369   Multiple forms of U2 snRNA coexist in the silk moth Bombyx mori. Insect Mol Biol.
370   2002;11(1):105–14.

371 21. Hinas A, Larsson P, Avesson L, Kirsebom LA, Virtanen A, Söderbom F. Identification of
372   the Major Spliceosomal RNAs in Dictyostelium discoideum Reveals Developmentally
373   Regulated U2 Variants and Polyadenylated snRNAs . Eukaryot Cell. 2006;5(6):924–34.

374 22. Korf GM, Botros IW, Stumph WE. Developmental and tissue-specific expression of U4
375   small nuclear RNA genes. Mol Cell Biol. 2015;8(12):5566–9.

376 23. Vazquez-Arango P, Vowles J, Browne C, Hartfield E, Fernandes HJR, Mandefro B, et al.
377   Variant U1 snRNAs are implicated in human pluripotent stem cell maintenance and
378   neuromuscular disease. Nucleic Acids Res. 2016;44(22):10960–73.

379 24. Leighton L, Bredy T. Functional Interplay between Small Non-Coding RNAs and RNA
380   Modification in the Brain. Non-Coding RNA. 2018;4(2):15.

381 25. Pagano JFB, Locati MD, Ensink WA, van Olst M, van Leeuwen S, de Leeuw WC, et al.
382   Maternal- and somatic-type snoRNA expression and processing in zebrafish
383   development. Submitted.

384 26. Locati MD, Pagano JFB, Ensink WA, van Olst M, van Leeuwen S, Nehrdich U, et al.
385   Linking maternal and somatic 5S rRNA types with different sequence-specific non-LTR
386   retrotransposons. RNA [Internet]. 2017;23(4):446–56. Available from:
387   http://rnajournal.cshlp.org/lookup/doi/10.1261/rna.059642.116

388 27. Locati MD, Pagano JFB, Girard G, Ensink WA, van Olst M, van Leeuwen S, et al.
389   Expression of distinct maternal and somatic 5.8S, 18S, and 28S rRNA types during
390   zebrafish development. RNA [Internet]. 2017;23(8):1188–99. Available from:
391   http://rnajournal.cshlp.org/lookup/doi/10.1261/rna.061515.117

392 28. Locati MD, Pagano JFB, Abdullah F, Ensink WA, van Olst M, van Leeuwen S, et al.
393   Identifying small RNAs derived from maternal- and somatic-type rRNAs in Zebrafish
394   Development. Genome [Internet]. 2018;gen-2017-0202. Available from:
395   http://www.nrcresearchpress.com/doi/10.1139/gen-2017-0202

396 29. Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J, et al. Ensembl 2018.
397   Nucleic Acids Res. 2018;46(D1):D754–61.

398 30. Kojima KK. Cross-Genome Screening of Novel Sequence-Specific Non-LTR
399   Retrotransposons: Various Multicopy RNA Genes and Microsatellites Are Selected as
400   Targets. Mol Biol Evol [Internet]. 2003;21(2):207–17. Available from:
401   https://academic.oup.com/mbe/article-lookup/doi/10.1093/molbev/msg235

402 31. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods.
403   2012 Apr;9(4):357–9.

404 32. McGrail JC, O'Keefe RT. The U1, U2 and U5 snRNAs crosslink to the 5' exon during
405   yeast pre-mRNA splicing. Nucleic Acids Res. 2008;36(3):814–25.

406 33. Kojima KK, Jurka J. A Superfamily of DNA Transposons Targeting Multicopy Small RNA
407   Genes. PLoS One. 2013;8(7):1–7.

408 34. Van Arsdell SW, Denison RA, Bernstein LB, Weiner AM, Manser T, Gesteland RF.
409   Direct repeats flank three small nuclear RNA pseudogenes in the human genome.
410   Cell. 1981;26(1 PART 1):11–7.

411 35. Matera AG, Wang Z. A day in the life of the spliceosome. Nat Rev Mol Cell Biol.
412   2014;15(2):108–21.

413 36. Lund E. Heterogeneity of human U1 snRNAs. Nucleic Acids Res. 1988;16(13):5813–26.

414 37. Lund E, Kahan B, Dahlberg JE. Differential control of U1 small nuclear RNA expression
415   during mouse development. Science (80-). 1985;229(4719):1271–4.

416

417

A

U1

```
M ATACTTACCTGGCAGGGGGAGATACCATGATCAAGAAGGTGGTTCACCCAGGGCGAGGCTTGGCCATTGCA
S . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . . CA . . . . . . . . .

M CT-CCGGCCACGCTGACCCCTGCGAATTCCCCAAATGTGGGAATCTCGACTGCATAATTTCTGGTAGTGG
S . . GT . . . . TGT . T . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

M GGGACTGCGTTCGCGCTCTCCCCTG
S . . . . . . . . . . . . . . . . . . . . . . . . . 11/165
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U2

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M ATCGCTTCTCGGCCTTTTGGCTAAGATCAAGTGTAGTATCTGTTCTTATCAGTTTAATATCTGATACGTG
S . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

M CCCTACCCGGGCACTATATATTAAATTGATTTTTGCAGCTGGGAGATGGGTTAGGGGCTTGCTCCACCCA
S . . . . . . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . G . . A . . . . . . . . . . AA . . . . . . . . . . . . . . GT . . .

M CTCCACGCATCGACCTGGTATTGCAGTACCTCCGGGAACGGTGCACTCATT
S . . . . . . . T . . . . . . C . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . C . CC . 13/191
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U4

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M ATTCTTTCGCAGAGGCAATATCATAGCCTATGAGGCCTAGCCGAGGCGTGATCATTGCTGGTTGAAAACT
S . GCT . . G . . . . T . . . G . . . G . . . . . . . . . . . . . . TT . . T . . . . . . . . . . C . . . T . . . . . AA . . . . . . . . .

M TTACCCAATACCCCGCCGAGATGGCTTGAAATACAGTCAGCTCTGGCAATTTTTGACAGCCTCCTAGAAG
S . . . . . . . . . . . . . . . . . . T . . C . A . . . . . . . . . T . . . G . . A . . . . . . . . . . . . . . . . G . T . . . GACAG . .

M G
S A 28/141
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U5

```
M ACAGCTCGAGTTTCTCTTCAATCACGCACAAATCTTTCGCCTTTTACTAAAGATTTCCGTGGGGAGGAAC
S GT . CACTC . TG . . T . TCCTC . GAT . . T . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . .

M TATTGTGAGTTTTGTTTATTTTTGGGTGCTCTGCTATCTGCAGAGCTG
S AC . ATGAGTA . AAAC . A . . . . . . T . A . . . C . . . . CGA . A . GC . G . GCT 48/118
```

U6

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M GTGCTCGCTACGGTGGCACATATACTAAAATTGGATCGATACAGAGAAGATTAGCATGGCCCCTGCGAAA
S . . . . . T . . . T . . . CA . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . C . .

M GGATGACACGCAAATCCGTGAAGCGCTCCATATTTCT
S . . . . . . . . . . . . . . . . . T . . . . . . . . . T . . . . . C . . . TA 11/118
```

B

Figure 1

Figure 2