

A dual ribosomal system in the zebrafish soma and germline

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Shah and colleagues take advantage of the presence of maternal and somatic ribosomes in zebrafish and confirm their differential expression during development. The authors **convincingly** show that ribosomes previously found expressed during oogenesis are also expressed in primordial germ cells and that hybrid maternal and somatic ribosomes are formed during development. The question of ribosome heterogeneity, the expression and function of maternal versus somatically provided ribosomes are of broad interest and this **fundamental** work sets new directions for future functional studies of this interesting phenomenon.

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Abstract

Protein synthesis during vertebrate embryogenesis is driven by ribosomes of two distinct origins: maternal ribosomes synthesized during oogenesis and stored in the egg, and somatic ribosomes, produced by the developing embryo after zygotic genome activation (ZGA). In zebrafish, these two ribosome types are expressed from different genomic loci and also differ in their ribosomal RNA (rRNA) sequence. To characterize this dual ribosome system further, we examined the expression patterns of maternal and somatic rRNAs during embryogenesis and in adult tissues. We found that maternal rRNAs are not only expressed during oogenesis but are continuously produced in the zebrafish germline. Proteomic analyses of maternal and somatic ribosomes unveiled differences in core ribosomal protein composition. Most nucleotide differences between maternal and somatic rRNAs are located in the flexible, structurally not resolved expansion segments. Our in vivo data demonstrated that both maternal and somatic ribosomes can be translationally active in the embryo. Using transgenically tagged maternal or somatic ribosome subunits, we experimentally confirm the presence of hybrid 80S ribosomes composed of 40S and 60S subunits from both origins and demonstrate the preferential in vivo association of maternal ribosomes with germline-specific transcripts. Our study identifies a distinct type of ribosomes in the zebrafish germline and thus presents a foundation for future explorations into possible regulatory mechanisms and functional roles of heterogeneous ribosomes.

Introduction

Ribosomes are macromolecular complexes composed of ribosomal RNAs (rRNAs) and proteins that are responsible for protein synthesis in the cell. Ribosomes of two different origins are present during embryogenesis. Large numbers of maternal ribosomes are synthesized during oogenesis and stored in the egg. Upon fertilization, maternal ribosomes are solely responsible for all translational activity during early embryogenesis (Bazzini et al. 2016 [↗](#); Hensey and Gautier 1997 [↗](#)). Maternal ribosomes are progressively degraded during embryogenesis and replaced by newly synthesized ribosomes (i.e., somatic ribosomes) that are produced after zygotic genome activation (ZGA) in the embryo (Heyn et al. 2017 [↗](#); Schramm and Bavister 1999 [↗](#)). In addition to their roles in translation (Bazzini et al. 2016 [↗](#); Cenik et al. 2019 [↗](#); Hensey and Gautier 1997 [↗](#); Noack Watt et al. 2016 [↗](#)), maternal ribosomes have been proposed to act as a source of pyrimidines required for the synthesis of new ribosomes (Liu et al. 2018b [↗](#)).

In the absence of transcription, the synthesis of new proteins in the early vertebrate embryo needs to be regulated at the post-transcriptional level. Several mechanisms have been described to regulate translation during early embryogenesis, including mRNA localization (Kloc and Etkin 2005 [↗](#); Lorenzo-Orts et al. 2024 [↗](#); Maegawa et al. 1999 [↗](#); Medioni et al. 2012 [↗](#)), binding of proteins or miRNAs to mRNAs (Giraldez et al. 2006 [↗](#)), and regulation of polyA tail length (Eichhorn et al. 2016 [↗](#); Lim et al. 2016 [↗](#); Liu et al. 2023 [↗](#); Subtelny et al. 2014 [↗](#)). While much effort has been devoted to understanding maternal mRNA regulation in the past, we have recently discovered that maternal ribosomes associate with a specific set of factors that bind to key sites on the ribosome and contribute to its repression and stability (Leesch and Lorenzo-Orts et al. 2023 [↗](#)). Thus, control of maternal ribosome function also plays an important role in translational regulation during embryogenesis.

Ribosomes have traditionally been considered homogeneous macromolecular complexes with no regulatory role in translation. However, several more recent studies proposed that ribosomes are heterogeneous in their composition (Ferretti et al. 2017 [↗](#); Komili et al. 2007 [↗](#); Mageeney and Ware 2019 [↗](#); O'Leary et al. 2013 [↗](#); Shi et al. 2017 [↗](#); Simsek et al. 2017 [↗](#); Xue et al. 2015 [↗](#)). Ribosome compositional heterogeneity is thought to be caused by variations in ribosome accessory factors, ribosome proteins, and ribosomal RNA, as well as by modifications of nucleotides and amino acids comprising the ribosome. For instance, distinct rRNAs have been reported in *Plasmodium berghei* ribosomes isolated from different life cycle stages (Gunderson et al. 1987 [↗](#); Waters et al. 1997 [↗](#)), and in ribosomes from *Xenopus* oocytes (Peterson et al. 1980 [↗](#)). Moreover, in *Drosophila*, ribosomes isolated from the germline and soma showed a different protein composition (Hopes et al. 2022 [↗](#)). Although heterogeneous ribosomes exist in various developmental and cellular contexts, the functional implications of ribosome heterogeneity remain controversial (Barna et al. 2022 [↗](#); Ferretti and Karbstein 2019 [↗](#)).

In zebrafish, maternal and somatic ribosomes differ in their composition (Locati et al. 2017a [↗](#), 2017b [↗](#)), which allows studying these specific ribosome types in an experimentally well-established vertebrate model organism. Maternal ribosomes differ in all four rRNA sequences (28S, 18S, 5.8S, and 5S) and originate from a different ribosomal DNA (rDNA) locus compared to somatic ribosomes (Locati et al. 2017a [↗](#), 2017b [↗](#)). Maternal rRNAs are transcribed during oogenesis from a single rDNA cluster located on chromosome 4 (Locati et al. 2017b [↗](#)), which is methylated and thus transcriptionally silenced in the soma (Ortega-Recalde et al. 2019 [↗](#)). Maternal rRNAs differ from somatic rRNAs in up to 14% of their sequence. However, the consequences of these differences on ribosome functions are not yet known. Furthermore, it remains unclear whether maternal and somatic ribosomes differ in protein composition and are able to form hybrid subunits, which would suggest functional compatibility among their subunits.

Here we analyze the expression, protein composition, and compatibility of maternal and somatic ribosomal subunits in zebrafish. Our results show that despite the differences in rRNA sequences, maternal and somatic ribosomes are found in polysomes indicating their translational activity and can form hybrid ribosomes containing subunits of both origins. Further, we identify germ cells as a cell type that continues to express maternal-type ribosomes throughout development, suggesting a potential functional importance in germ cell-specific translation.

Results

Expression of rRNA variants across development and cell types in zebrafish

To investigate the expression of maternal and somatic rRNA variants during zebrafish development, we analyzed total RNA during the first five days of embryogenesis. Consistent with previous findings (Locati et al. 2017a [\[1\]](#), 2017b [\[2\]](#)), we observed that maternal rRNAs originating from the maternal rDNA locus on chromosome 4 (GRCz11; 4: 77,564,140 – 77,555,053) were the sole rRNA variants present in the egg and during early stages of embryogenesis before zygotic genome activation (ZGA). Following ZGA, somatic rRNAs started to be transcribed from the somatic rDNA locus on chromosome– 5 (GRCz11; 5: 827,807 – 819,029). The levels of somatic rRNAs increased progressively and reached the same level as maternal rRNAs between 20 to 30 hours post-fertilization (hpf). By 5 days post-fertilization (dpf), more than 98% of the detected rRNAs were of somatic origin, reflecting the transition from maternal to somatic ribosomes (**Fig. 1A** [\[3\]](#) and **1B** [\[4\]](#)).

Although most ribosomes are of somatic origin by day 5 of development, maternal rRNA variants may be expressed in specific cell types or tissues beyond oocytes. To investigate this, we employed a PCR-based assay that could discriminate between maternal and somatic rRNA variants based on the amplification of highly variable Expansion Segments (ESs) in 18S and 28S RNAs (**Supplementary Fig. S1A** [\[5\]](#)). To confirm the applicability of the PCR-based assay, we recapitulated the transition from maternal to somatic 18S rRNA (**Supplementary Fig. S1B** [\[6\]](#) and **S1C** [\[7\]](#)). While in most adult tissues a single DNA band corresponding to somatic 18S and 28S rRNAs was detected, testis samples showed evidence for the presence of both somatic and maternal rRNAs (**Supplementary Fig. S2** [\[8\]](#)). Next-generation sequencing (NGS) experiments using total RNA from testes dissected from five different adult male zebrafish revealed variable levels of maternal and somatic rRNAs, with maternal rRNA variants accounting for 2% to 27% of the total rRNAs (**Fig. 1C** [\[9\]](#)). Together, these results suggest that both maternal and somatic ribosomes are present in the male germline.

To gain deeper insights into the expression of maternal rRNAs in the germline, we re-analyzed total RNA sequencing data from whole embryos and larvae, as well as published total RNA sequencing data from FACS-sorted primordial germ cells (PGCs) during larval development up to 10 dpf (Redl et al. 2021 [\[10\]](#)). PGCs, which are the precursors of the adult germline, showed persistently high levels of maternal rRNAs compared to somatic larval tissues throughout 10 dpf (**Fig. 1D** [\[11\]](#)). Internal transcribed spacer (ITS) sequences are removed from the nascent 47S rRNA and act as a marker for ribosome biogenesis. Consistent with a potential role for maternal ribosomes in the germline, expression analysis of maternal ITS1 (**Fig. 1A** [\[12\]](#)), indicated active transcription of maternal rRNAs in PGCs, but not in the soma, starting from 3 dpf (**Supplementary Fig. S3** [\[13\]](#)); long before the production of early-stage oocytes between 10-25 dpf in juveniles (Dranow et al. 2016 [\[14\]](#); Takahashi 1977 [\[15\]](#)).

Despite our evidence for persistent expression of the rDNA locus on chromosome 4 in the zebrafish germline, for simplicity and consistency with previous publications, we will continue to refer to the rRNAs, the subunits they compose, and the ribosomes generated from this locus as “maternal” throughout this manuscript.

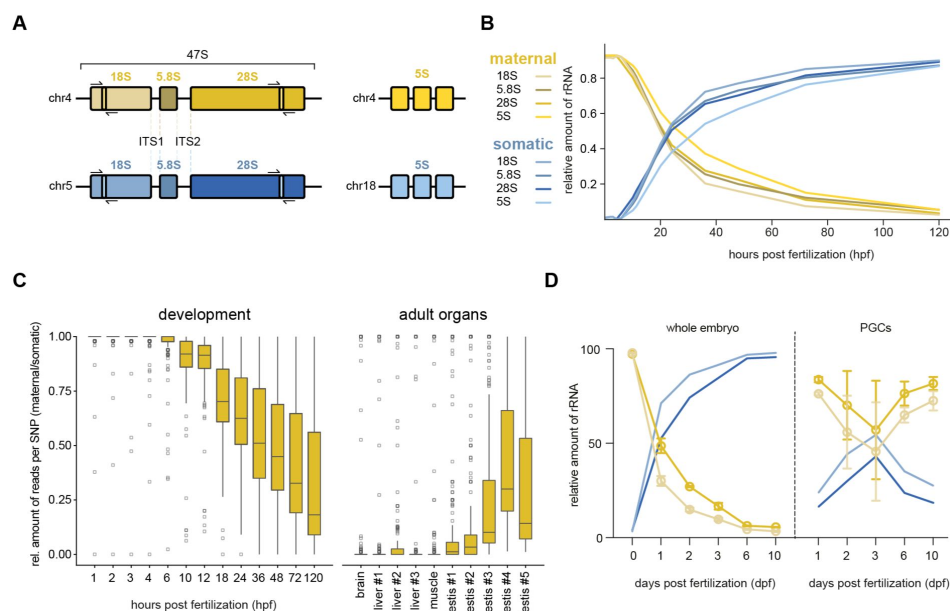


Figure 1

Expression of maternal and somatic rRNA variants in zebrafish

A) Organization of maternal (yellow) and somatic (blue) rDNA genes in the zebrafish genome. The 5S rRNA genes are encoded separately, while 18S, 5.8S, and 28S are derived from a single 47S pre-rRNA transcript containing additional spacer sequences (ITS1 and ITS2 (internal transcribed spacers)) that are removed in several processing steps. Expansion segments (ES3S and ES31L) are indicated by lighter-colored boxes and PCR primer icons (see **Supplementary Fig. S1A**). **B)** Relative expression levels of the four rRNAs (28S, 5.8S, 18S, and 5S), comparing maternal (yellow) and somatic (blue) variants. **C)** Fraction of maternal versus somatic rRNAs, obtained by quantifying reads with variant-specific SNPs (single nucleotide polymorphisms) during the first five days of development, in adult somatic organs, and in testes of 5 different males. **D)** Expression of maternal and somatic rRNA variants in whole embryo/larva lysate and FACS-sorted PGCs. ES, expansion segment. FACS, fluorescence activated cell sorting.

Recent interrogation of human cancer biopsies in The Cancer Genome Atlas (TCGA) (Liu et al. 2018a) has similarly shown differential expression of rRNA variants (Rothschild et al. 2023). To test whether zebrafish maternal-type rRNAs might also be re-expressed in immortalized cells or during tumorigenesis, we analyzed cDNAs from several cultured zebrafish cell lines and excised adult tumors (Berghmans et al. 2005; Driever and Rangini 1993; Heilmann et al. 2015; Patton et al. 2005; Paw and Zon 1999; Perez et al. 2018). 18S rRNA compositional analysis in the assayed *ex vivo* or neoplastic contexts showed no evidence of reactivated maternal rRNA (Supplementary Fig. S4A and S4B). Similarly testing regenerating fin blastema through 5 days post amputation indicates dedifferentiated cells losing morphology and re-entering the cell cycle (Pfefferli and Jaźwińska, 2015) do not express maternal rRNA (Supplementary Fig. S4C).

Maternal and somatic ribosomes differ in protein composition

Maternal and somatic ribosomes have so far only been shown to differ in their rRNAs. To investigate potential differences in protein composition, we performed proteomic analyses of ribosomes isolated at different times during embryogenesis and compared the expression of core ribosomal proteins (RPs) (Supplementary Table S1). We chose time points containing either only maternal ribosomes (egg, 1 hpf, 3 hpf and 6 hpf) (Leesch and Lorenzo-Orts et al., 2023) or only somatic ribosomes (120 hpf) (Fig. 1B). We detected 8 RPs with more than one paralog associated to maternal or somatic ribosomes during embryogenesis, namely Rps8 (eS8), Rps17 (eS17), Rps26 (eS26), Rps27 (eS27), Rpl2 (P2), Rpl5 (uL18), Rpl7 (uL30) and Rpl22 (eL22) (Fig. 2A). Moreover, alternative isoforms were evident for Rps5 (uS7), Rps18 (uS13), Rps19 (eS19) and Rpl9 (uL6) (Fig. 2A).

To gain further insights into the expression patterns of these alternative RPs, we analyzed previously published RNA-seq datasets covering different stages of oogenesis, embryogenesis, and adult tissues (Cabrera-Quio et al. 2021; Noda et al. 2022; Pauli et al. 2014) (Fig. 2B, Supplementary Fig. S5). While only one protein variant was predominantly present (more than 10-fold difference in expression level) for all RPs (Fig. 2A), mRNAs encoding for Rps17, Rps27.1/Rps27.2, Rpl2/Rpl21 and Rpl5a/Rpl5b were expressed at more similar levels during development and in adult tissues (Fig. 2B, Supplementary Fig. S5). In some cases, one RP paralog was associated with purified ribosomes at all stages while the other was ribosome-bound only at specific times. Examples include Rpl5b and Rpl22, which were associated with ribosomes throughout embryogenesis, whereas their paralogs Rpl5a and Rpl221, respectively, only showed increased ribosome incorporation at 5 dpf, correlating with the expression of somatic rRNA variants (Fig. 2B and 2C). This proteomic analysis of maternal and somatic ribosomes during embryogenesis provided evidence for RP composition differences in assembled ribosomes at different times of development, but did not reveal a single RP paralog pair that mirrored the switch between maternal and somatic rRNAs.

Nucleotide differences between maternal and somatic rRNAs are predominantly located in structurally non-resolved flexible areas of the ribosome

rRNAs provide the ribosome with a structural framework and form many of its core functional sites. Since maternal and somatic ribosomes contain different rRNAs and RPs, we mapped the sites of nucleotide differences onto the previously obtained cryo-EM structure of the maternal ribosome at 6 hpf (Leesch and Lorenzo-Orts et al. 2023) (Fig. 3A, Supplementary Fig. S6A). We chose the 6 hpf time-point since it contains exclusively maternal ribosomes (and thus maternal rRNAs) that have already lost the repressive dormancy factors that are initially bound at 1 hpf, and embryos already contain a larger amount of polysomes than at 1 hpf (Leesch and Lorenzo-Orts et al. 2023),

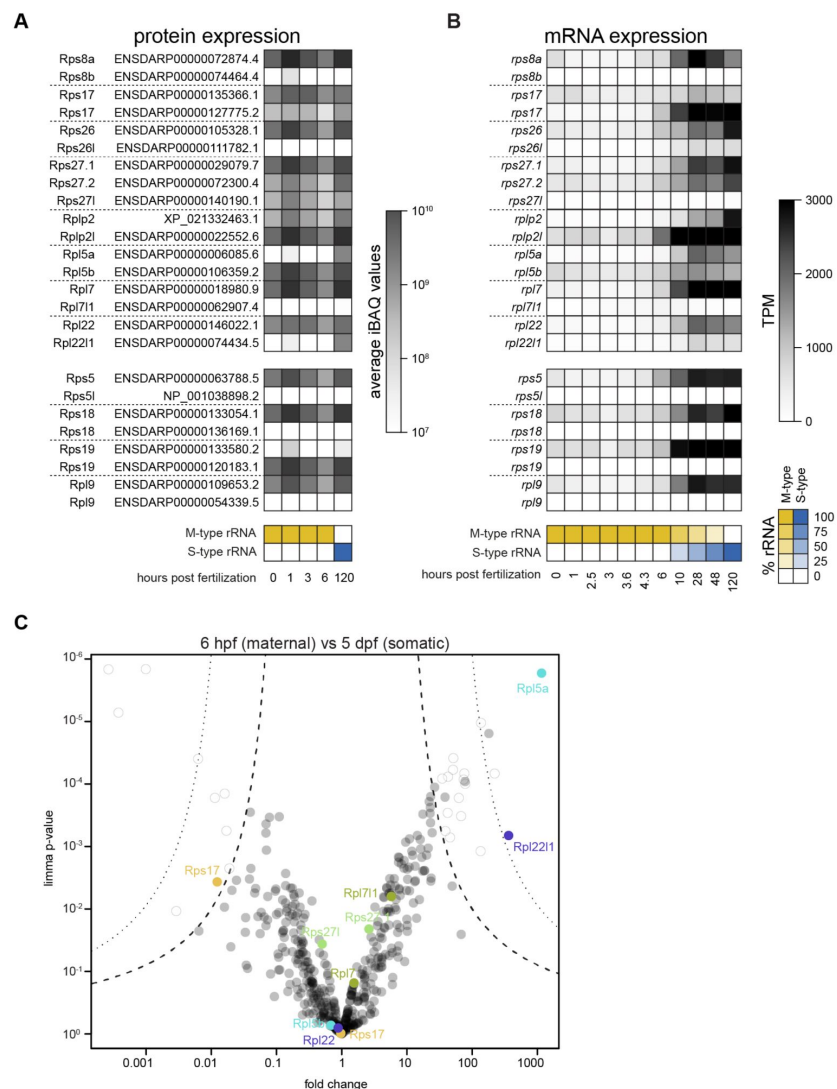


Figure 2

Expression of alternative ribosomal core proteins in zebrafish development

A) Heatmap of protein expression (normalized IBAQ values) for variant ribosomal protein pairs (paralogs, top; alternative isoforms, bottom) associated with zebrafish ribosomes at different developmental stages. Below, relative levels of maternal (yellow) and somatic (blue) rRNA variants at these developmental stages are indicated separately. **B)** Heatmap of mRNA expression of paralogs and alternative isoforms for ribosomal protein variants during embryogenesis. See also **Supplementary Fig. S5**. The relative levels of maternal (yellow) and somatic (blue) rRNA variants at these developmental stages are indicated in the scheme below the heat map. **C)** Volcano plot based on mass spectrometry data showing fold enrichment of proteins in the ribosome fraction of 5 dpf larvae versus 6 hpf embryos ($n = 3$ for each time point). Pairs of ribosomal protein variants with significantly different associations are shown in different shades. Non-significant but detected variants are shown in green. Permutation-based false discovery rates (FDRs) are shown as dotted (FDR < 0.01) and dashed (FDR < 0.05) lines. All significantly enriched or depleted proteins are listed in **Supplementary Table S1**. TPM, transcripts per million. IBAQ, intensity Based Absolute Quantification.

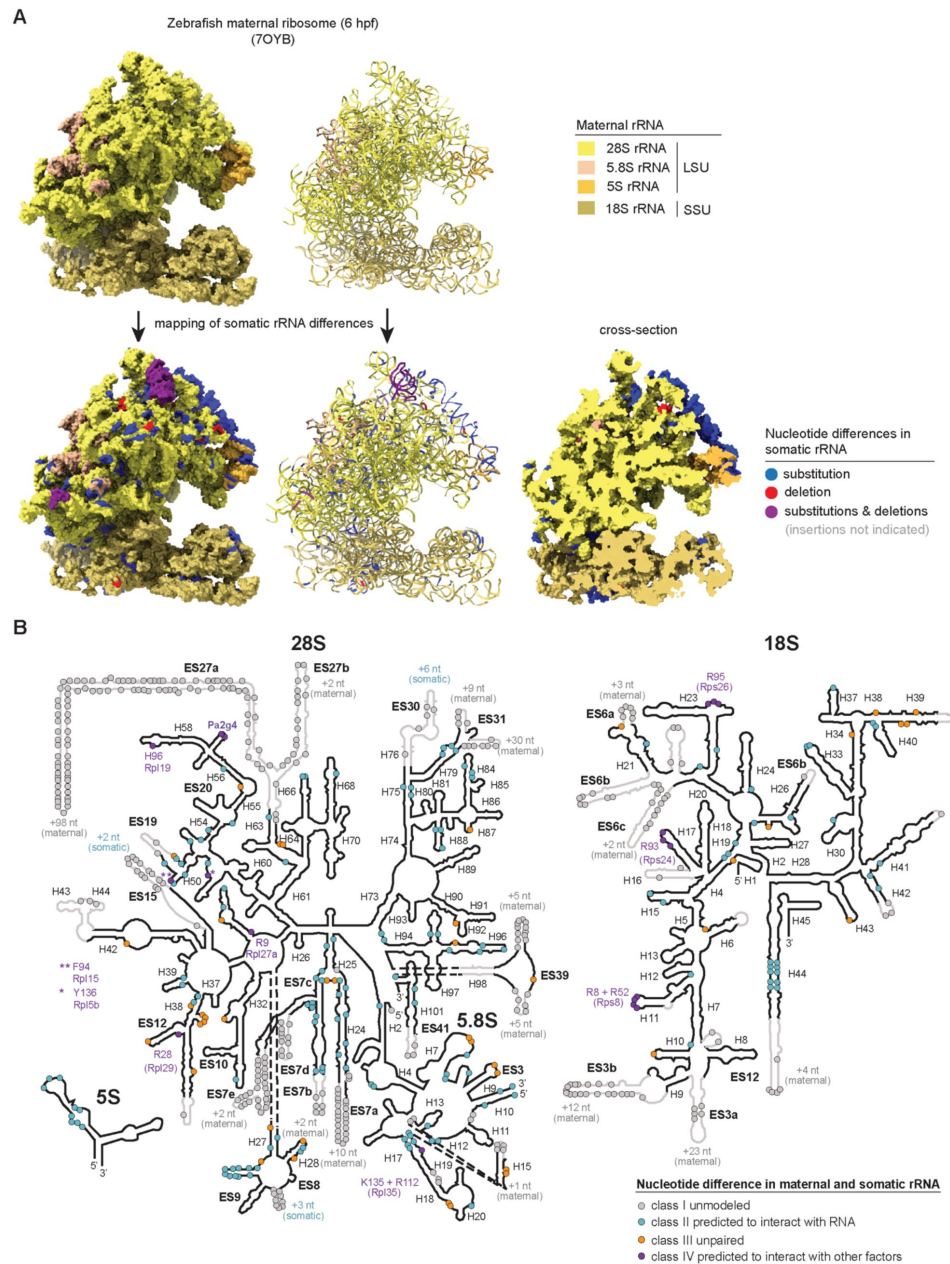


Figure 3

Location of sequence and structural differences in rRNA variants

A) Map of the maternal zebrafish ribosome isolated from 6 hpf embryos (PDB-7OYB; Leesch and Lorenzo-Orts et al., 2023). Maternal rRNA sequences are shown in surface representation (left) and cartoon representation (right) in shades of yellow. Locations of nucleotide differences in zebrafish somatic rRNAs are highlighted at the bottom (see legend for color code). A cross-section through the model is shown in the right, revealing that most of the sequence differences are located on the surface and not in the inside of the ribosome's core. **B)** Sequence differences in maternal and somatic rRNA variants mapped onto rRNA secondary structure predictions derived from R2DT (Sweeney et al., 2021). 18S rRNA, 5.8S and 5S: structural predictions for *Danio rerio* somatic rRNAs; 28S rRNA: structural prediction for the *Homo sapiens* 28S rRNA, with the ES regions adjusted based on modelling of the zebrafish sequence in the Vienna RNAfold package (Lorenz et al., 2011). Circles indicate positions of nucleotide differences between the maternal and somatic rRNAs. Regions not modeled in the cryo-EM structure obtained from 6 hpf zebrafish embryos due to poor density are shown in gray.

As previously suggested (Locati et al. 2017a) the majority of sequence differences between maternal and somatic rRNAs are located in expansion segments (ESs), which are flexible RNA elements on the surface of the ribosome and thus often not resolved by structural methods (Fig. 3A and 3B, Movies 1-3). In fact, only 27% of the nucleotides that differ between maternal and somatic rRNAs were visible in our maternal ribosome structure. In several instances, complementary nucleotide differences were predicted to be present in Watson-Crick base-pairs (e.g. H17, H44, ES27; Fig. 3B), thus likely preserving rRNA interactions and secondary structure (Supplementary Table S2).

Functionally important nucleotides tend to be conserved throughout evolution. We therefore also analyzed whether the differences between maternal and somatic rRNAs corresponded to evolutionarily conserved or more variable nucleotides. We found that nucleotides that differed between zebrafish maternal and somatic rRNAs were depleted from conserved regions (characterized by low Shannon entropies; Schmitt and Herzel 1997) and instead enriched in areas with high degrees of variability at the single nucleotide level across evolution (high Shannon entropies) (Supplementary Fig. S6B and S6C). Together, our analyses reveal that the majority of nucleotide differences between maternal and somatic ribosomes are located in evolutionary less conserved regions outside of the structurally well-resolved areas of the ribosome.

Formation of hybrid ribosomes comprising maternal and somatic subunits

To investigate whether maternal and somatic ribosomal subunits may interact and form 80S “hybrid” ribosomes, we focused our structural analyses on rRNA sequence differences and alternative ribosomal proteins in the contact regions between 40S and 60S subunits, termed intersubunit bridges (see Table 1). 40S and 60S subunits interact through 17 bridges (B1a-eB14) (Anger et al. 2013; Ben-Shem et al. 2011; Melnikov et al. 2012). Most of these bridges are formed by RNA-RNA or RNA-protein contacts, with the exception of B1b/c, which is formed by the contact of two ribosomal proteins, Rps18 (uS13) and Rpl11 (uL5). Five bridges are specific for eukaryotic ribosomes (eB8, eB11, eB12, eB13, and eB14). On the RNA level, there are sequence differences between maternal and somatic rRNAs in intersubunit bridges B3-B6, eB13, and in expansion segments ES31L, ES41L, and ES6S (Fig. 3B), which form bridges eB8, eB11, eB12 and eB14, respectively (Table 1). In addition to rRNA sequence differences, one ribosomal protein, Rps8 (eS8), known to form an intersubunit bridge (Tamm et al. 2019) has a paralogous RP and both are expressed in early zebrafish embryogenesis (Fig. 2A). However, in our data, Rps8a was more abundant at the protein and mRNA level in both maternal and somatic ribosomes, suggesting no difference in RP paralog usage at the intersubunit bridge between maternal and somatic ribosomes (Fig. 2A and 2B).

To directly investigate the possibility of hybrid ribosome formation *in vivo*, 24 hpf zebrafish larvae were used since maternal and somatic rRNA variants are present at equal levels at this developmental stage (Fig. 1B, Supplementary Fig. S1B). To assess whether maternal ribosomes can be found in polysomes at this developmental stage, we performed polysome gradients of total lysates, which showed peaks for the 40S and 60S ribosomal subunits, a large 80S (monosome) peak and several polysome peaks (Fig. 4A). Besides detecting maternal and somatic rRNA types in the fraction corresponding to 40S, 60S and 80S subunits, we identified both rRNA types in translationally active polysomes (Fig. 4B; see Supplementary Fig. S1B for the specificity of the primers), suggesting that both maternal and somatic ribosomes support translation at this stage of embryogenesis.

To achieve selective labeling of maternal and somatic ribosomal subunits, FLAG-tagged Rpl10a was specifically expressed during oogenesis via the oogenesis-specific promoter *sycp* (synaptonemal complex protein) (Tg(Mat-RiboFLAG), ensuring the restricted incorporation into

inter subunit bridge	40S SSU		60S LSU		sequence differences in rRNA regions
	protein	RNA	protein	RNA	
B1a	uS19 (Rps15)			H38a	yes (H38a)
B1b/c	uS13 (Rps18)		uL5 (Rpl11)		-
B2a		H44		H69	no
B2b		H45		H64 + H66	no
B2c		H24 + H27		H66	no
B3		H44		H70	yes (H44)
B4	uS15 (Rps13)	H20		H34	yes (H34)
B5		H44	uL14 (Rpl23)	H71	yes (H44)
B6		H44		H62 + H64	yes (H44)
B7a		H23		H68	no
B7b/c		H23	uL2 (Rpl8)		no
B8		H5	uL14 (Rpl23)		no
eB8	eS1 (Rps3a)			ES31L	yes (ES31L)
eB11	eS8 (Rps8)			ES41L	yes (ES41L)
eB12		ES6S	eL19 (Rpl19)		yes (ES6S)
eB13		H10	eL24e(Rpl24)		yes (H10)
eB14		H27, H45, H44	eL41 (Rpl41)		yes (H27, H44, H45)

Table 1

Structural differences of intersubunit bridge regions in maternal and somatic ribosomes

Intersubunit bridges (listed in the first column) are connections between 40S SSU (composed of the 18S rRNA and 33 RPs) and 60S LSU (composed of the 5S, 5.8S, 28S rRNAs, and 46 RPs). Bridges composed of rRNA sequences with differences between maternal and somatic types are marked. H, helix. ES, expansion segment.

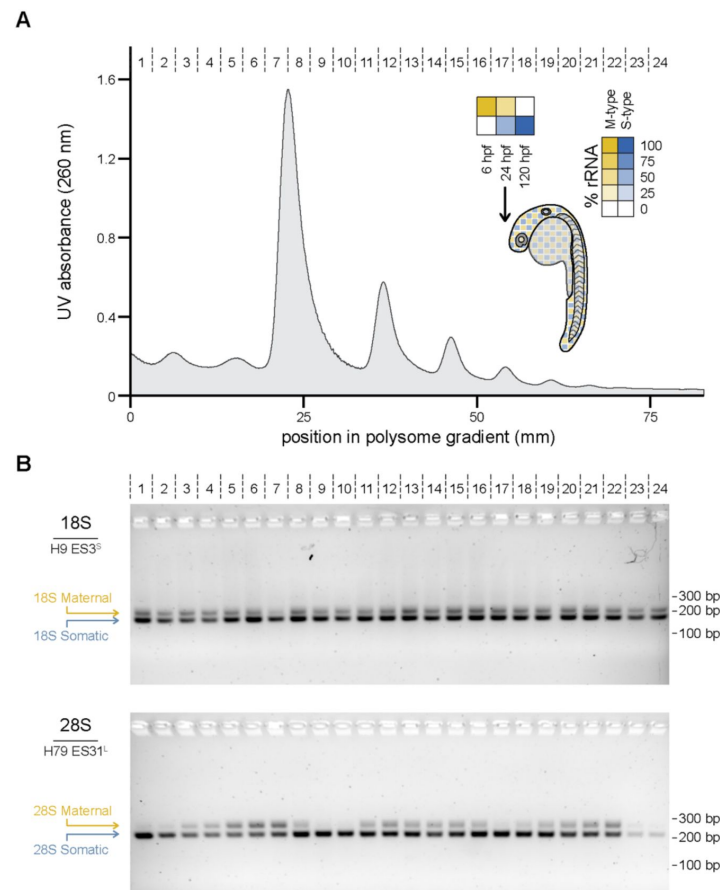


Figure 4

Maternal and somatic ribosomal subunits are translationally active in 24 hpf embryos

A) Representative polysome profile with continuous UV absorbance (A₂₆₀) reading from sucrose density gradients containing lysate from 24 hpf zebrafish embryos for which the relative levels of each rRNA variant is shown in the inset. Position in the gradient and collected fractions are indicated. **B)** Gel electrophoretic analysis of PCR-based detection of maternal and somatic small (18S) and large (28S) rRNA variants in the individual fractions indicated in (A). See **Supplementary Fig. S1B** for the specificity of the primers and **Supplementary Table S3** for lengths of PCR products from maternal and somatic rRNAs. bp (base pair), H (helix), ES (expansion segment).

maternal ribosomes. In contrast, a male-contributed transgene using a ubiquitously expressed promoter *ubi* (ubiquitin b) (Tg(Som-RiboFLAG)) enabled specific labeling of somatic ribosomes (**Supplementary Fig. S7** [↗](#), **S8** [↗](#) and **S9** [↗](#)).

To validate our experimental setup (**Fig. 5A** [↗](#)), we first performed FLAG-immunoprecipitation (FLAG-IP) experiments using maternal and somatic FLAG-tagged Rpl10a embryo lysates treated with EDTA to dissociate 80S ribosomes into individual subunits. By analyzing the relative abundance of maternal and somatic 28S rRNA in the input and IP fractions using RT-qPCR, we confirmed the specificity of our tagging, precipitation, and detection approaches (**Fig. 5B** [↗](#)).

To test whether hybrid ribosomes form *in vivo*, we performed a second FLAG-IP experiment in the presence of cycloheximide (CHX), a well-established translation inhibitor known to stabilize translating ribosomes. This allowed us to assess the presence of maternal or somatic small subunits (SSUs) in the IP fraction of the tagged maternal or somatic LSUs (**Fig. 5C** [↗](#)). Intriguingly, we observed similar levels of maternal and somatic SSUs in the IP fractions independently of whether maternal or somatic tagged LSUs had served as baits. These findings provide direct experimental evidence for the *in vivo* formation of hybrid ribosomes, supporting the compatibility between large and small ribosomal subunits of different origins.

Co-enrichment of maternal ribosomes with PGCs and PGC-specific mRNAs

Building on our observation that maternal rRNAs are continuously expressed in PGCs (**Fig. 1D** [↗](#), **Supplementary Fig. S3B** [↗](#)) and that maternal ribosomes are actively translating in 1-day-old zebrafish embryos, we reasoned that maternal ribosomes should be present in PGCs and may be translating PGC-specific transcripts. To test these hypotheses, we injected *in vitro* transcribed *eGFP* mRNA fused to the *nanos3-3'UTR* into the 1-cell stage embryo. The *nanos3-3'UTR* stabilizes the transcript only in PGCs, enabling PGC-specific *eGFP* translation at 1 dpf (**Fig. 6A** [↗](#) and **6B** [↗](#)).

To confirm that PGCs maintain a higher ratio of maternal versus somatic ribosomes at 1 dpf (**Fig. 1D** [↗](#)), we used RT-qPCR to assess the ratio of maternal and somatic rRNA variants in FACS-isolated *eGFP*-positive PGCs and non-*eGFP* expressing somatic cells. As expected, a higher abundance of maternal 18S and 28S rRNA variants were detected in the isolated PGCs compared to somatic cells, confirming the persistence of maternal ribosomes at higher levels in germline cells (**Fig. 6C** [↗](#)). To directly test whether maternal ribosomes associate with PGC-specific transcripts and thus may contribute to germ cell-specific translation, we performed a RNA immunoprecipitation (RIP) assay using biotinylated *eGFP-nanos3-3'UTR* mRNAs extracted from 1-day-old cycloheximide-treated larvae. This approach allowed us to analyze the pool of ribosomes bound to this specific PGC-localized mRNA. Notably, we observed a significant enrichment of the maternal ribosomes compared to somatic ribosomes associated with biotin-labeled PGC-localized mRNAs (**Fig. 6D** [↗](#)). However, this apparent level of enrichment was similar to the level of maternal ribosomes in isolated PGCs (**Fig. 6C** [↗](#)), providing no evidence towards selectivity of maternal ribosomes for germ cell-specific transcripts. Nevertheless, our results reveal an association between maternal ribosomes and PGCs, and thus germline-specific transcripts.

Discussion

Previous studies have revealed that zebrafish ribosomes of maternal and somatic origin are composed of two distinct sets of rRNAs (Locati et al. 2017a [↗](#), 2017b [↗](#)). While one set of rRNAs was thought to be exclusively present in maternal ribosomes, our PCR-based and RNA-seq analyses indicate that these rRNAs are not only transcribed during oogenesis, but are also present in PGCs and the male germ line. Translation in PGCs is tightly regulated and differs from that in the soma, as these cells are specified before the onset of zygotic genome activation (ZGA) and

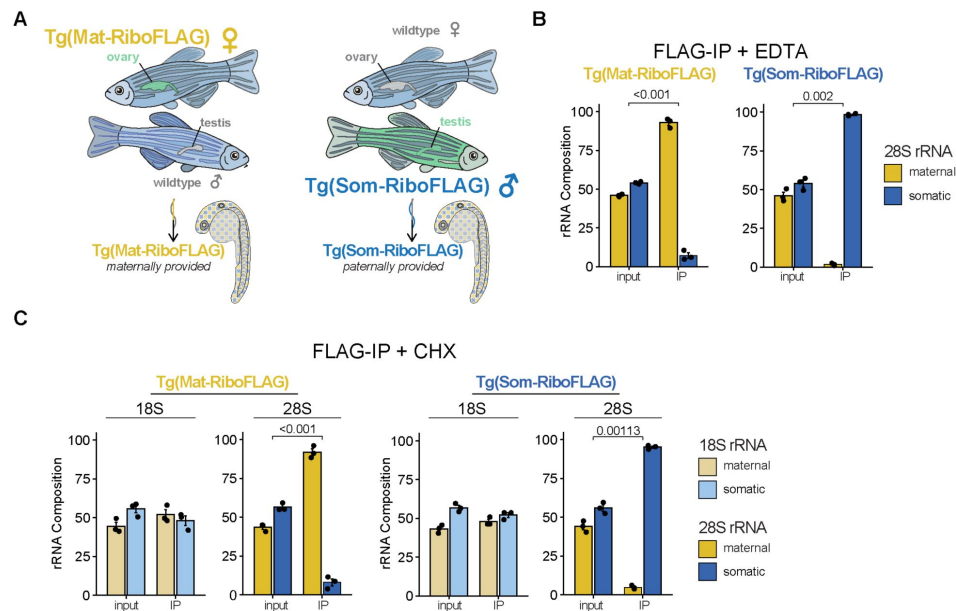


Figure 5

In vivo evidence for hybrid ribosome formation in 1 day post-fertilization embryos

A) Scheme summarizing the generation of embryos with tagged ribosomes by crossing Tg(Mat-RiboFLAG) females to wildtype males and Tg(Som-RiboFLAG) males to wildtype females (see Methods). **B)** RT-qPCR analysis of the relative amounts of maternal and somatic 28S rRNAs detected in input or eluate (IP) fractions of a FLAG-IP experiment, which used EDTA-treated lysates containing either tagged maternal (Tg(Mat-RiboFLAG)) or somatic (Tg(Som-RiboFLAG)) 60S subunits as input. **C)** RT-qPCR analysis of the relative amounts of maternal and somatic 28S and 18S rRNAs detected in input or eluate (IP) fractions of a FLAG-IP experiment. Cycloheximide (CHX) was added during lysis of embryos containing either tagged maternal (Tg(Mat-RiboFLAG)) or somatic (Tg(Som-RiboFLAG)) 60S subunits. Monosome fractions from RNase-treated lysates were used as input. In (B) and (C), three biological replicates from independent natural crosses were used, and significance was calculated by t-test (if not indicated, p-value > 0.05).

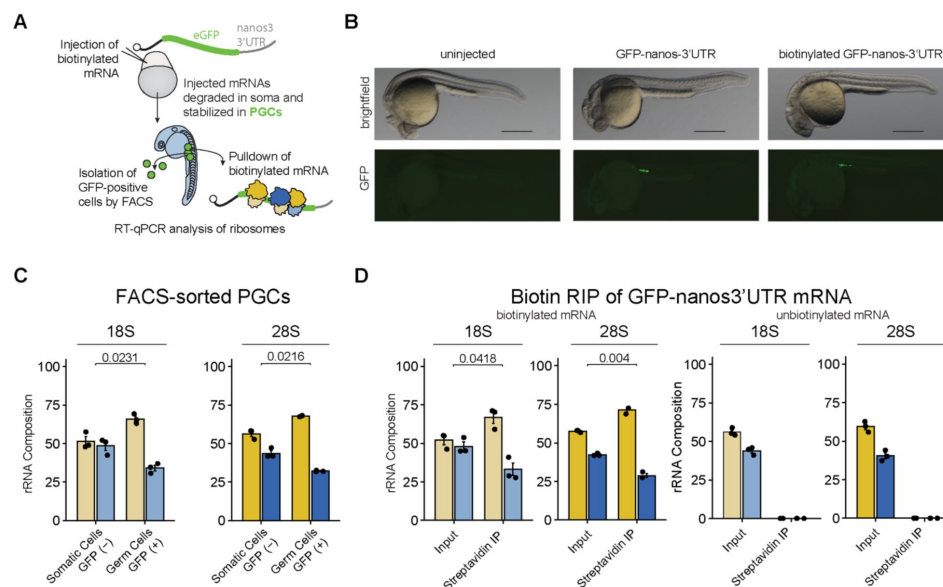


Figure 6

Enrichment of PGC-localized mRNAs with maternal ribosomes at 1 day post-fertilization

A) Schematic of the experimental strategy. eGFP-nanos3'UTR containing mRNA was injected into 1-cell stage zebrafish embryos from which two different experimental approaches were used to investigate the ratio of maternal and somatic ribosomes in PGCs and to test which ribosomes are bound to PGC-localized mRNAs. **B**) Brightfield and eGFP images of 1 day post-fertilization embryos, previously injected with only water, eGFP-nanos3'UTR mRNA, or biotinylated eGFP-nanos3'UTR mRNA. Scale bars indicate 500 μ m. **C**) Ratio of maternal (yellow) versus somatic (blue) 18S and 28S rRNA in FACS-isolated eGFP-positive (PGCs) and eGFP-negative (somatic) cells analyzed by RT-qPCR. **D**) Ratio of maternal (yellow) versus somatic (blue) 18S and 28S variants associated with biotinylated eGFP-nanos3'UTR mRNAs isolated by RIP (RNA immunoprecipitation). Non-biotinylated eGFP-nanos3'UTR mRNA served as control. Non-specific (background) amplification was determined using IPs from wild-type (uninjected) lysate and was set to zero. In (C) and (D), three biological replicates from independent natural crosses were used, and significance was calculated by t-test (if not indicated, p-value > 0.05).

contain specific maternal mRNAs (Dranow et al. 2016 [DOI](#); Johnson et al. 2011 [DOI](#); Yu et al. 2016 [DOI](#)). Through at least 10 dpf, both inherited and zygotically generated maternal ribosomes constitute the major ribosome type in PGCs. Therefore, maternal ribosomes may comprise a distinct set of ribosomes that drive translation in the germline - a finding that may have broad implications for understanding germline determination and development in zebrafish.

Heterogeneous rRNA sequences have been described in various organisms, including fungi, invertebrates (Dimarco et al. 2012 [DOI](#)), *Xenopus*, and humans (Pasolini et al. 2006 [DOI](#); Vierna et al. 2013 [DOI](#)). However, whether ribosome heterogeneity translates into specialized ribosomal functions is still a matter of scientific debate. Apart from *Plasmodium* species (Gunderson et al. 1987 [DOI](#); Li et al. 1997 [DOI](#); Waters et al. 1997 [DOI](#)) and the 5S rRNA in *Xenopus* (Peterson et al. 1980 [DOI](#)), zebrafish embryos are the only known system that contains two distinct ribosomes composed of different sets of rRNAs. Differential expression of rRNA variants between tissues may indicate the ability of rRNA variant ribosomes to have distinct functions. Therefore, zebrafish embryogenesis, especially germline versus soma studies, provides a powerful system to study ribosome-mRNA interactions in the context of the functional consequences of ribosome heterogeneity. However, the cell-to-cell variability in their abundance requires future techniques to address these questions at the single cell level.

Importantly, we observed that at 1 dpf, when maternal and somatic ribosomes are present at similar levels, both types of ribosomes associate with polysomes and are thus likely involved in translation. Consistent with the role of maternal ribosomes in supporting early development, translation by maternally deposited ribosomes is essential and increases during embryogenesis before the onset of ribosome biogenesis (Bazzini et al. 2016 [DOI](#); Hensey and Gautier 1997 [DOI](#); Leesch and Lorenzo-Orts et al. 2023 [DOI](#)). Notably, in nematode worms lacking zygotic production of ribosomes (Cenik et al., 2016) and in zebrafish mutants with impaired ribosome biogenesis (Bielczyk-Maczyńska et al. 2015 [DOI](#); Noack Watt et al. 2016 [DOI](#)), maternal ribosomes have been shown to be sufficient to sustain embryonic development.

If both maternal and somatic ribosomes are capable of translation, an obvious question is why a dual ribosomal system has evolved in zebrafish. One hypothesis is that maternal ribosomes may favor the translation of certain transcripts. Fully addressing this question would require swapping the maternal and somatic rRNA locus, a challenging experiment in zebrafish. Given that the major sequence differences between maternal and somatic rRNAs are in expansion segments, transcript specificity (if any) may be caused by solvent-exposed surfaces of maternal and somatic ribosomes. Our finding that 80S “hybrid” monosomes composed of 40S and 60S subunits of different origins are readily formed during zebrafish embryogenesis argues against maternal and somatic ribosomes acting as distinct entities.

As previously mentioned, though many sequence differences exist between the two, compensatory mutations of Watson-Crick base-pairs may preserve rRNA secondary structure in maternal and somatic ribosomes. For instance, both strands of helix 12 (H12) of the 28S rRNA differ in maternal and somatic ribosomes, with the somatic variant containing a U (U68) and the maternal sequence containing a C (C68). In the opposite strand, the maternal variant encodes a compensatory G73, whereas the somatic variant contains A73, thereby maintaining the Watson-Crick interaction (U-A in the somatic rRNA, C-G in the maternal rRNA). The sarcin-rich domain (SRD, or sarcin-rich loop, SRL) (Anger et al. 2013 [DOI](#); Khatter et al. 2015 [DOI](#)) is a functionally important site of the ribosome, and contains two nucleotide differences ($U_{\text{som}}^{3716}/C_{\text{mat}}^{3864}$ and $A_{\text{som}}^{3738}/G_{\text{mat}}^{3886}$) located at the helix that forms the base of the SRL stem-loop. It is unclear if this represents a molecular difference among either ribosomes' capacity to anchor elongation factors during tRNA translocation (Shi et al. 2012 [DOI](#); Szewczak et al. 1993 [DOI](#)). Other functionally important regions like the GTPase association center (GAC), which acts as a binding platform for elongation factors and ribosome rescue factors (Uchiyumi and Kominami 1994 [DOI](#)), were not resolved in our maternal ribosome cryo-EM map in the region containing three nucleotide differences between maternal

and somatic 28S RNAs ($A_{\text{som}}1558/C_{\text{mat}}1573$, $U_{\text{som}}1560/C_{\text{mat}}1575$ and $U_{\text{som}}1565/C_{\text{mat}}1580$). Therefore, future studies will be required to directly compare the structures of maternal and somatic ribosomes, though our work already reveals that the vast majority of nucleotide differences is located at the surface on the ribosome and thus unlikely to alter for example ribosomal catalysis.

An alternative and non-exclusive hypothesis is that the maternal rDNA locus may be involved in PGC fate and sex determination in zebrafish. Laboratory strains of zebrafish lack sex chromosomes and, unlike mammals, rely on the transfer of specific maternal factors for germ cell development. Several reports suggest a role for the maternal rDNA locus in sex determination, with specific changes (such as demethylation and amplification on extrachromosomal circles) occurring during oocyte expansion and being associated with feminization (Breit et al. 2020 [DOI](#); Dranow et al. 2013 [DOI](#); Ortega-Recalde et al. 2019 [DOI](#); Tao et al. 2020 [DOI](#)).

Another possibility is that the distinct set of rRNAs affects ribosome stability, as it has been suggested that maternal ribosomes are degraded more rapidly than their somatic counterparts (Locati et al. 2017b [DOI](#)). A recent study proposed that ribosome ubiquitination plays a role in targeting and degrading maternal ribosomes during zebrafish embryogenesis (Ugajin et al., 2023 [DOI](#)). Additionally, one of the expansion segments, ES4L, is located on the ribosome surface and is formed by the 3' end of the 5.8S rRNA and the 5' end of the 28S rRNA. This 3' half of the 5.8S rRNA has previously been suggested to play a role in ribosome degradation (Locati et al., 2018), and therefore this region could potentially serve as a target for degradation of maternal ribosomes.

Taken together, our study describes a distinct set of ribosomes present in the zebrafish germline. The presence of two distinct ribosomes during zebrafish embryogenesis provides an experimentally accessible system for future studies of ribosome specialization and defines a potentially novel mechanism of translational regulation in the zebrafish germline.

Materials and Methods

Zebrafish lines and husbandry

Zebrafish (*D. rerio*) experiments in the Pauli lab were conducted according to Austrian and European guidelines for animal research and approved by local Austrian authorities (animal protocols for work with zebrafish: GZ 342445/2016/12 and MA 58-221180-2021-16), and zebrafish lines in the Calo lab were housed in AAALAC-approved facilities and maintained according to protocols approved by the Massachusetts Institute of Technology Committee on Animal Care (CAC). Zebrafish transgenic lines containing maternally or somatically tagged ribosomes were generated as part of this study and are described below. In vivo samples were allocated randomly to the experiment and treated equally.

TLAB fish, generated by crossing zebrafish AB with the natural variant TL (Tupfel Longfin), served as wild-type zebrafish for experiments in the Pauli lab (**Figures 1B** [DOI](#), **2** [DOI](#), **3** [DOI](#), and **Supplementary Figures S2** [DOI](#), **S5** [DOI](#) and **S6** [DOI](#)). All experiments in the Calo Lab were performed in the AB/Tübingen (TAB5/14) zebrafish genetic background (**Figures 4** [DOI](#), **5** [DOI](#), **6** [DOI](#), and **Supplementary Figures S1B** [DOI](#), **S4** [DOI](#), **S7** [DOI](#), **S8** [DOI](#) and **S9** [DOI](#)). Fish were raised according to standard protocols (28 °C; 14:10 h light:dark cycle).

Zebrafish embryo and tissue collection

Zebrafish embryos were collected at the indicated times post-fertilization, and staged according to (Kimmel et al. 1995 [DOI](#)).

Zebrafish AB9 fibroblasts, originally derived from an adult caudal fin (ATCC CRL-2298), were cultured on cell culture-treated 10 cm plates (Genesee Scientific, 25-500), collected using trypsin (Gibco, 25200072) and standard cell culture procedures. AB9 cells were grown at 28 °C with 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM, Genesee Scientific, 25-500) supplemented with 10% fetal bovine serum (FBS, Gemini Bio-products) and 1% Penicillin/Streptomycin (Gibco, 10378-016).

Zebrafish ZMEL melanoma cells, originally derived from Tp53^{M214K/M214K} tumors expressing human oncogenic BRAF^{V600E} were cultured and collected using the same protocols as AB9 cells. ZMEL cells were a kind gift from the laboratory of Dr. Leonard Zon (Boston Children's Hospital).

Zebrafish ZF4 fibroblasts, originally derived from a 1 dpf embryo (ATCC CRL-2050), were cultured on cell culture-treated 10 cm plates (Genesee Scientific, 25-400), collected using trypsin (Gibco, 25200072) and standard cell culture procedures. ZF4 cells were grown at 28 °C with 5% CO₂ in a 1:1 mixture of Dulbecco's Modified Eagle Medium and Ham's F-12 media (DMEM/F12, Genesee Scientific, 25-503) supplemented with 10% fetal bovine serum (FBS, Gemini Bio-products) and 1% Penicillin/Streptomycin (Gibco, 10378-016).

For all cell lines, cells from a 50-75% confluent 10 cm plate were collected and either flash frozen or directly lysed in TRIzol.

Zebrafish tumors used in this study were derived from the genotypes Tg(mitfa: GNAQ^{Q209L}); Tp53^{M214K/M214K} (referred to as GNAQ melanoma), Tg(BRAF^{V600E}); Tp53^{M214K/M214K} (referred to as BRAF melanoma), and Tp53^{M214K/M214K} (referred to as MPNST, malignant peripheral nerve sheath tumor) and excised using standard protocols approved by the Committee on Animal Care at MIT.

Adult zebrafish were monitored and euthanized before tumors (3-4 mm in diameter) were excised with a sterile scalpel and placed into a 35 mm dish. Tumors were incubated with 2 mL of Dissection Media [Dulbecco's Modified Eagle Medium (DMEM, Genesee Scientific, 25-500), 1% Penicillin/Streptomycin (Gibco, 10378-016), and 75 µg/mL of Liberase (Roche, 5401020001)], manually disaggregated with a sterile razor blade, and let sit at room temperature until a cellular slurry formed. Tumors were further broken down by trituration with an additional 5 mL of Wash Solution [1x PBS (Genesee Scientific, 25-508), 1% Penicillin/Streptomycin (Gibco, 10378-016), and 10% fetal bovine serum (FBS, Gemini Bio-products)]. Cells were passed through a 40 µm strainer (Falcon, 352340), pelleted for 2 min at 500 xg, washed twice with 1x PBS, pelleted, and lysed in TRIzol.

Generation of transgenic fish lines

Plasmid construction was based on the Tol2/Gateway zebrafish kit (Kwan et al. 2007 [\[1\]](#)). The p5e-ubi entry clone was a generous gift from the laboratory of Dr. Leonard Zon (Boston Children's Hospital). The p5e-sycp plasmid was cloned by amplifying the sycp promoter from genomic DNA before using Gateway BP Clonase II to insert the gel-selected product into pDONRP4-P1R (#219) using previously described primers (Gautier et al. 2013 [\[2\]](#)). The pMe-3xFLAG-eGFP-Rpl10a plasmid was cloned by stitching together PCR products containing 3xFLAG, eGFP, and Rpl10a coding sequence (amplified from 1 dpf cDNA) via Gibson Assembly (Gibson et al. 2009 [\[3\]](#)), then amplified using gateway primers before using Gateway BP Clonase II (ThermoFisher Scientific, 11789020) to clone the insert product. The pMe-3xFLAG-eGFP-Rpl10a-430-3'UTR plasmid was similarly cloned. The p3e-polyA entry clone (#302) and pDestTol2 (#393) were used from the Tol2Kit in conjunction with the above plasmids and LR Clonase II (ThermoFisher Scientific, 11791020) to generate ubi:eGFP-3xFLAG-Rpl10a:polyA and sycp:eGFP-3xFLAG-Rpl10a-430-3'UTR:polyA and are referred to as Tg(Som-RiboFLAG) and Tg(Mat-RiboFLAG) respectively.

To generate mosaic transgenic fish lines, a 1 nL mixture of plasmid DNA (40 ng/μL) and capped-mRNA encoding Tol2 Transposase (20 ng/μL) was loaded into needles pulled from glass capillary tubing (Warner, 64–0766) and injected into one-cell-stage embryos using a pico-liter injector (Warner Instruments, PLI90A). Mosaic founders were crossed with wild-type animals to generate a stable line.

F3 and F4 embryos were used for imaging and experiments. Brightfield images and eGFP images were acquired on a Leica M205 FCA fluorescence stereo microscope. Images were taken with the same settings for all embryos.

FACS isolation of primordial germ cells (PGCs)

One-cell-stage zebrafish embryos were injected with 1–2 nL of capped *eGFP-nanos3'UTR* mRNA using a pico-liter injector (Warner Instruments, PLI90A) and needles pulled from glass capillary tubing (Warner, 64–0766). At 24 hpf, embryos were sorted for eGFP expression, dechorionated using 1 mg/mL pronase (Pronase from *Streptomyces griseus*, Cat# 000000010165921001, Sigma-Aldrich), and washed with 1x PBS (Genesee Scientific, 25–508).

80 eGFP-positive embryos were incubated in a 5 mL solution of TrypLE Express (ThermoFisher Scientific, 12605036) and 0.003% Tricaine (ethyl-3-aminobenzoate-methanesulfonate) for 60 min at 31 °C in a Thermomixer (Eppendorf, Thermomixer R) set to 100 rpm, followed by trituration using a glass Pasteur pipette until tissues were digested into single cells. Cells were passed through a 40 μm strainer (Falcon, 352340), pelleted for 5 min at 500 xg at room temperature, and washed with 1x PBS. Cell pellets were resuspended in 1x PBS supplemented with 5% fetal bovine serum (FBS, Gemini Bio-products). Using a BD FACS Aria cell sorter, approximately 1,200 eGFP-expressing cells (primordial germ cells) and 1,200 non-eGFP-expressing cells (somatic cells) were sorted directly into TRIzol.

Additions to polysome gradient protocols

To obtain a better resolution of the low molecular weight fractions, 24 hour post-fertilization embryos were batch deyolked according to the previously published protocol ([Link et al. 2006](#)). While 200 to 250 embryos were used per sample for non-deyolked samples, 500 embryos were used per gradient for deyolked embryos. The deyolking buffer was supplemented with 1x protease inhibitors (EDTA-free, Roche) and 0.1 μg/mL cycloheximide. To avoid DNA contamination in the higher molecular weight fractions, obtained larval lysates were treated with 1:125 Turbo DNase (QIAGEN, 79254) according to the manufactures protocol and incubated for 10 min at 28 °C.

To isolate RNA after fractionation, 400 μL from each collected fraction were transferred into a Safe-lock Eppendorf tube, 1000 μL TRIzol (ThermoFisher Scientific, 15596-018) reagent were added and samples were vortexed for 10 s immediately. The following steps were performed according to the standard TRIzol based RNA isolation protocol.

Western blotting of polysome fractions

400 μL of each fraction was first mixed with 700 μL water to dilute sucrose. Sodium deoxycholate and TCA were added to a final concentration of 0.08% and 20% respectively. Samples were vortexed and incubated overnight at –20 °C and centrifuged at 20,000 xg at 4 °C for 30 min. Protein pellets were washed twice with 2 volumes of cold (–20 °C) 100% acetone, then pelleted again. After acetone was removed, protein pellets were air dried, resuspended in 100 μL Laemmli buffer, sonicated for 5 min (30 s on, 30 s off), and boiled for 5 min, before running a portion on a 4–20% Tris-glycine polyacrylamide gel (Invitrogen, XV04205). Transfer to a PVDF membrane previously activated with methanol, was run in cold Tris-glycine transfer buffer (1x Tris-glycine, 20% methanol) at 80 V for 80 min. After transfer, membranes were blocked in 5% milk in PBST (1x PBS with 0.1% Tween-20) for 1 h and blotted with primary antibodies (1: 2000 anti-FLAG, Millipore

Sigma, F3165, and 1:500 anti-Rpl7, ThermoFisher Scientific, A300-741A) overnight at 4 °C. Membranes were washed with PBST and blotted with secondary antibodies (1:10,000 anti-mouse, Invitrogen, 32230, or 1:10,000 anti-rabbit, Invitrogen, 32260) for 1 h at room temperature. All blots were sequentially probed SuperSignal West Femto (ThermoFisher Scientific, 34096) was used to develop the blot and a Bioanalytical Imaging System model c500 (Azure Biosciences) was used for imaging.

Isolation of zebrafish embryos for total RNA time course

Embryos were snap-frozen in liquid nitrogen and stored at -80 °C. To maintain a uniform genetic background, all embryos for the time course experiment were collected from the same fish stock.

Mapping of somatic rRNA differences onto the maternal rRNA structure

Nucleotide sequence alignments between maternal and somatic zebrafish rRNAs were generated in Clustal Omega (<https://www.ebi.ac.uk/jdispatcher/msa/clustalo>) (Sievers et al. 2011) for each of the rRNA classes (28S, 18S, 5.8S and 5S), and pairwise nucleotide sequence differences were identified. Nucleotide positions that differed in the somatic compared to the maternal version of the rRNA (see **Supplementary Table S2**) were highlighted via ChimeraX-1.6.1 (Goddard et al. 2018; Pettersen et al. 2021; Meng et al. 2023) on the rRNA structural model of the maternal 6 hpf ribosome (PDB: 7OYB, Leesch and Lorenzo-Orts et al., 2023). Moreover, 2D rRNA maps were downloaded from R2DT (Sweeney et al., 2021; <https://rnacentral.org/r2dt>) (somatic 18S, 5.8S, 5S rRNA 2D model: *Danio rerio*; 28S rRNA 2D model: *Homo sapiens*, with expansion segments that differed most in zebrafish modeled via the Vienna RNAfold package (Lorenz et al., 2011)), and used as basis for mapping and classifying maternal vs somatic nucleotide differences. Human rRNA 2D maps highlighting nucleotide position-specific Shannon Entropy values based on the Eukarya phylogeny were downloaded from Ribovision2 (<https://ribovision2.chemistry.gatech.edu/>; Bernier et al., 2014), and adapted by highlighting the positions of maternal-vs-somatic nucleotide differences in zebrafish (**Supplementary Fig S6B** and **S6C**).

RNA extraction from TRIzol

Homogenized lysate was mixed with 0.2 mL chloroform per mL of TRIzol, vortexed, and centrifuged for 15 minutes at 16,000 xg at 4 °C. The upper aqueous phase containing RNA was mixed with an equal volume of 100% ethanol, vortexed, processed with a Zymo column (RNA Clean and Concentrator, Zymo, R1014) including on-column DNase treatment, and eluted in 13 µL RNase-free water.

cDNA synthesis

cDNA was synthesized using SuperScript IV VILO Master Mix (ThermoFisher Scientific, 11766050). For each sample, an 8 µL reaction composed of 6.4 µL RNA, 0.8 µL 10x ezDNase Buffer, and 0.8 µL ezDNase enzyme was incubated at 37 °C for 5 min before centrifuging and placing on ice. Then, 3 µL of SuperScript IV VILO Master Mix or No RT Control and 4 µL of RNase-free water was added to each reaction, followed by incubation at 25 °C for 10 min, 60 °C for 10 min, and 85 °C for 5 min.

Detection of maternal and somatic rRNA variants by PCR

Primer sequences and annealing temperatures are listed in **Supplementary Table S3**. For certain PCRs, due to the high G/C content of rRNAs and therefore of the corresponding cDNA, standard PCR conditions were adapted to these more complex templates. RNA and cDNA were isolated as described above. The PCR reaction differed from the manufacturer's protocol in the addition of 1.3x Q5® Hot Start High-Fidelity (from a 2x MasterMix, New England Biolabs M0494)

and additionally 1 M betaine (Sigma-Aldrich, B0300). The PCR conditions were modified according to the standard protocol by extending of the elongation time up to 90s in each PCR cycle and a final elongation step of 15 min.

Detection of maternal and somatic rRNA variants by RT-qPCR

The synthesized cDNA was diluted 5-1000 fold with RNase-free water, depending on the input material, and used in a 10 μ L RT-qPCR reaction composed of 5 μ L 2x KAPA SYBR FAST qPCR Master Mix (Roche, KK4600), 2.5 μ L cDNA, and 0.25 μ M of each primer. Reactions were arrayed into a 384-well reaction plate or a 96-well reaction and measured with a Light Cyclers 480 II Real-time PCR Machine: 95 °C for 30 s, 35 cycles of 95 °C for 5 s, 60 °C for 15 s and 68 °C for 15 s. Three biological replicates, each from independent natural crosses, were measured in triplicate. No reverse-transcriptase samples were included as negative controls. Background amplification was determined using IPs from wild-type (uninjected) lysate. qPCR results with cycle numbers higher than the 'background control' were determined as 'non-specific amplification' and thus set to zero. Relative fold differences of specific targets were calculated using the $2\Delta\text{Ct}$ method. Statistical analyses were performed using Student's unpaired t-test where $p < 0.05$ was considered significant.

In vitro transcription of mRNA and biotin labeling

To generate the RNA for microinjections, 2 μ g of a construct encoding eGFP-nanos3'UTR was digested using NotI-HF (New England BioLabs, R3189S) for 1 h. The linearized product was purified according to manufacturer specifications via gel selection using NucleoSpin (Macherey-Nagel, 740588.50). 500 ng of DNA was used in an overnight IVT reaction using mMACHINE mMACHINE T7 ULTRA Transcription Kit (Invitrogen, AM1345). The product was treated with TURBO DNase for 30 min. RNA was poly-A-tailed using the provided tailing reaction and samples were run on agarose gels to confirm sizes. RNA was purified using Zymo RNA Clean and Concentrator columns (Zymo Research, R1016) and concentration was measured using a Nanodrop ND-1000 Spectrophotometer (ThermoFisher Scientific).

End labeling of RNA was achieved using previously described methods (Wang et al. 2007 [\[3\]](#)). Briefly, 20 pmol of in vitro transcribed mRNA (approximately 10 μ g) was subjected to labeling with 1 nmol of pCp-Biotin (Jena Bioscience, NU-1706-BIO) in a 20 μ L reaction comprising 5 μ L DMSO, 2 μ L 10 mM ATP, 1 μ L T4 RNA Ligase 1 (NEB, M0437M), 1 μ L SuperAseIn (Invitrogen AM2694), and 2 μ L T4 RNA Ligase Buffer at 25 °C for 2 h. A no-labeling reaction was similarly incubated with the reaction components lacking pCp-Biotin. All mRNAs were purified using Zymo RNA columns and adjusted to 200 ng/ μ L.

Isolation of zebrafish ribosome subunits by immunoprecipitation

Embryos were washed twice with 500 μ L of ice-cold Subunit Buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, and 1 mM DTT. As much buffer as possible was removed before samples were either immediately processed or flash frozen and stored at -80 °C.

400 μ L of ice-cold Subunit Lysis Buffer (Subunit Buffer with 1% Triton X-100, 1x cOmplete-EDTA-free protease inhibitor (Sigma, 11873580001), and 20 mM EDTA) was added to either fresh or frozen tissue. Keeping tubes on ice, a pre-cooled 1 mL syringe attached to a 26-gauge needle was used to shear samples by slowly triturating material for 20-30 strokes until no visible tissue remained. Lysates were incubated on ice for 5 min, then cleared by centrifugation at 10,000 $\times$ g for 10 min at 4 °C.

Cleared S10 fractions were adjusted to 500 μ L with Subunit Lysis Buffer and overlaid onto 2 mL of a 1 M sucrose cushion prepared in Subunit Buffer in a 15 $\times$ 51 mm 3 mL thickwall polycarbonate ultracentrifuge tube (Beckman Coulter, 349622) on ice. Ribosomal subunits were pelleted using a

TLA100.3 rotor (Beckman Coulter, 349490) at $\sim 300,000$ xg at 4 °C for 2 h. Supernatants were carefully poured off and pellets were resuspended by horizontal rotation on ice for at least 30 min in 235 μ L of Subunit Buffer. Resuspended subunits were incubated with 2 μ L of anti-FLAG antibody (Millipore Sigma, F3165-.2MG) for 1 h at 4 °C. Then, 14 μ L of equilibrated Dynabeads Protein G (ThermoFisher Scientific, 10003D) were added and incubated at 4 °C for at least 4 h. Beads were subsequently collected using a magnetic rack, washed with Subunit Buffer three times, and resuspended in TRIzol-LS reagent (Invitrogen, 10296028).

Isolation of zebrafish monosomes by immunoprecipitation

Embryos were washed twice with 500 μ L of ice-cold Ribosome Buffer [20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 1 mM DTT]. As much buffer as possible was removed before samples were either immediately processed or flash frozen and stored at -80 °C.

200 μ L of ice-cold Ribosome Lysis Buffer [Ribosome Buffer containing 1% Triton X-100, 1x cComplete-EDTA-free protease inhibitor (Sigma, 11873580001), and 100 μ g/mL cycloheximide] was added to either fresh or frozen tissue. Keeping tubes on ice, a pre-cooled 1 mL syringe attached to a 26-gauge needle was used to shear samples by slowly triturating material for 20-30 strokes until no visible tissue remained. Lysates were incubated on ice for 5 min, cleared by centrifugation at 10,000 xg for 10 min at 4 °C, then treated with 1 unit per embryo of RNase T1 (ThermoFisher, EN0541). After 90 min of rotating at room temperature, 2 units per embryo of SuperAseIn (Invitrogen, AM2694) were added to stop the reaction.

Cleared and digested S10 fractions were adjusted to 250 μ L with Ribosome Lysis Buffer and overlaid onto a continuous 10-50% (w/v) sucrose gradient prepared in Ribosome Buffer containing 100 μ g/mL cycloheximide. Gradients were generated using a BioComp Gradient Master device and 14 $\times$ 89 mm ultracentrifuge tubes (Seton Scientific, 7030). Gradients were centrifuged in a SW41 Ti rotor (Beckman Coulter, 331362) at 35,000 rpm ($r_{\max}$ of 210,000 xg) for 150 min at 4 °C followed by analysis and fractionation using a BioComp Gradient Station coupled to a Model Triax™ Flow Cell detector (FC-2). Approximately 1500 μ L from the monosome fraction were manually collected.

The resulting monosomes were incubated with 2 μ L of anti-FLAG antibody (Millipore Sigma, F3165-.2MG) for 1 h at 4 °C. Then, 14 μ L of equilibrated Dynabeads Protein G (ThermoFisher Scientific, 10003D) were added and incubated at 4 °C for at least 4 h. Beads were subsequently collected using a magnetic rack, washed three times with Ribosome Buffer containing 100 μ g/mL cycloheximide, and resuspended in TRIzol-LS reagent (Invitrogen, 10296028).

RIP of biotinylated mRNAs

One-cell-stage zebrafish embryos were injected with 1-2 nL of either biotinylated or non-biotinylated *eGFP-nanos3'UTR* mRNA. At 24 hpf, embryos were dechorionated using pronase (Pronase from Streptomyces griseus, Cat# 10165921001, Sigma-Aldrich) and checked for eGFP expression.

Approximately 400 eGFP-positive embryos were washed twice with 500 μ L of ice-cold Ribosome Buffer [20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 1 mM DTT]. As much buffer as possible was removed before samples were immediately processed.

400 μ L of ice-cold Ribosome Lysis Buffer [Ribosome Buffer containing 1% Triton X-100, 1x cComplete-EDTA-free protease inhibitor (Sigma, 11873580001), and 100 μ g/mL cycloheximide] was added to fresh tissue. Keeping tubes on ice, a pre-cooled 1 mL syringe attached to a 26-gauge needle was used to shear samples by slowly triturating material for 20-30 strokes until no visible tissue remained. Lysates were incubated on ice for 5 min, then cleared by centrifugation at 10,000 xg for 10 min at 4 °C.

Cleared S10 fractions were adjusted to 500 μ L with Ribosome Lysis Buffer and overlaid onto 2 mL of a 1 M sucrose cushion prepared in Ribosome Buffer containing 100 μ g/mL cycloheximide in a 15 $\times$ 51 mm 3 mL thickwall polycarbonate ultracentrifuge tube (Beckman Coulter, 349622) on ice. Ribosomes were pelleted using a TLA100.3 rotor (Beckman Coulter, 349490) at 80,000 rpm ($r_{\max}$ of 346,000 $\times g$) for 2 h at 4 °C. Supernatants were carefully poured off and pellets were resuspended by horizontal rotation on ice for at least 30 min in 235 μ L of Ribosome Buffer with 100 μ g/mL cycloheximide and 5 μ L SuperAseIn (Invitrogen AM2694).

Streptavidin magnetic beads (Invitrogen, 65001) were washed twice with Ribosome Buffer, then added to resuspended ribosomes, and incubated with rotation overnight at 4 °C. Beads were magnetically collected, washed three times with Ribosome Buffer containing 100 μ g/mL cycloheximide and resuspended in TRIzol LS reagent (Invitrogen, 10296028).

Data availability

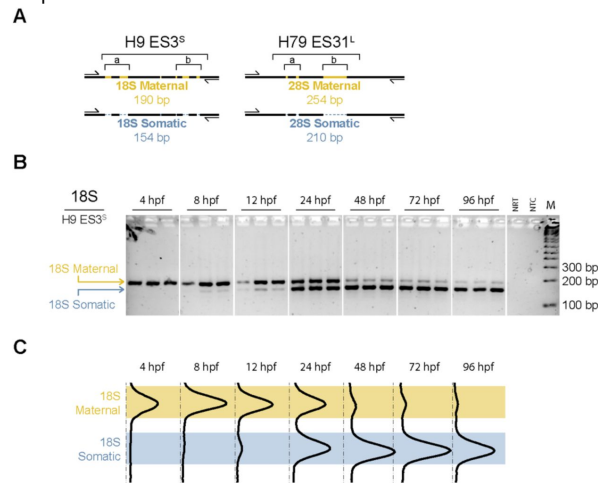
Mass spectrometry proteomics data of isolated zebrafish ribosomes from 0, 1, 3 and 6 hpf has been published previously (Leesch and Lorenzo-Orts et al. 2023 [DOI](#)) and is accessible via the PRIDE partner repository with the dataset identifier PXD026866. Mass spectrometry proteomics data of isolated zebrafish ribosomes from 120 hpf (5 dpf) has been deposited to the ProteomeXchange Consortium via the PRIDE partner repository and will be made accessible upon publication. RNA-Seq data of total RNA (not ribosomal RNA depleted) was deposited to GEO and will be made accessible upon publication.

Supplementary Figures

Supplementary Figure S1

Detection of maternal and somatic rRNAs in embryonic development

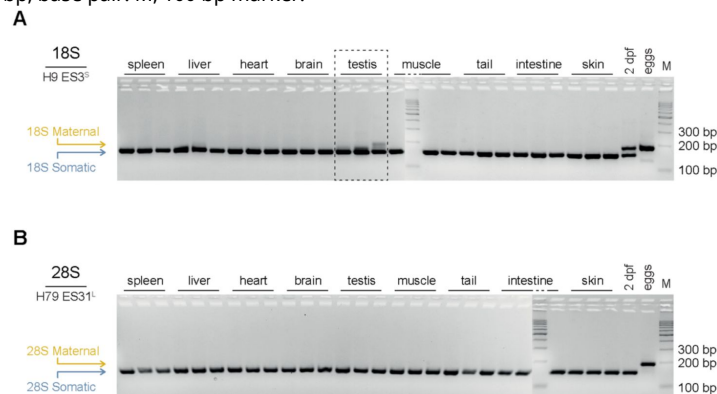
A) Schematic representation of the rRNA expansion segments (ES) depicted in **Fig. 1A** which are targeted for a PCR-based fragment length polymorphism (FLP) assay. Either ES3S (helical regions *H9a* and *H9b*) in the 18S rRNA or ES31L (helical regions *H79a* and *H79b*) in the 28S rRNA are PCR amplified using a single primer pair (indicated by primer icons) detecting both maternal and somatic rRNA variants in the same reaction. Resulting PCR products differ in size due to the different lengths of ES regions in each rRNA. **Supplementary Table S3** contains the relevant PCR conditions and primer sequences. **B)** Gel electrophoretic analysis of PCR-based detection of maternal and somatic rRNA variants at the indicated developmental times. For each developmental time assayed, three individual embryos, each from an independent natural cross comprising one wildtype female and one wildtype male, were used for RNA extraction and cDNA synthesis. **C)** Averaged ($n=3$) line profiles of densitometry scans (ImageJ) from each lane of the gel shown in (B). The regions representing signal from maternal and somatic 18S rRNA PCR FLP are indicated and used for relative quantification in **Fig. 1B**. bp, base pair. NRT, no RT control. M, 100 bp marker.



Supplementary Figure S2

Analysis of rRNA variant expression in different tissues

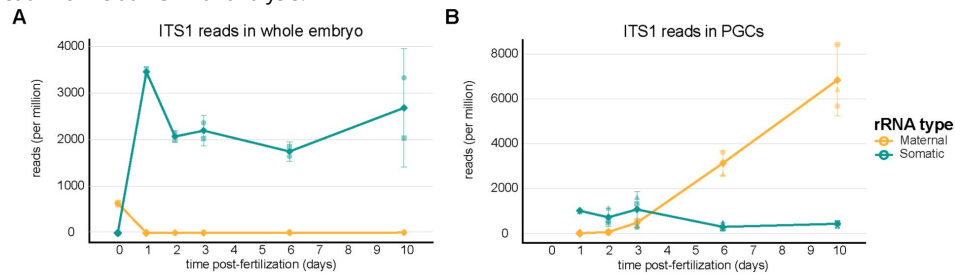
Gel electrophoretic analysis of PCR-based detection of maternal and somatic **A)** 18S rRNA and **B)** 28S rRNA variants in different adult zebrafish tissues ($n=3$, each). Positive control reactions using 2 dpf and egg cDNAs are included for reference. See **Supplementary Fig. S1** and **Supplementary Table S3** for indicated lengths of PCR products from maternal and somatic rRNAs. H, helix. bp, base pair. M, 100 bp marker.



Supplementary Figure S3

Transcription of rRNA variants in zebrafish and primordial germ cells (PGCs) from 1 through 10 days post fertilization

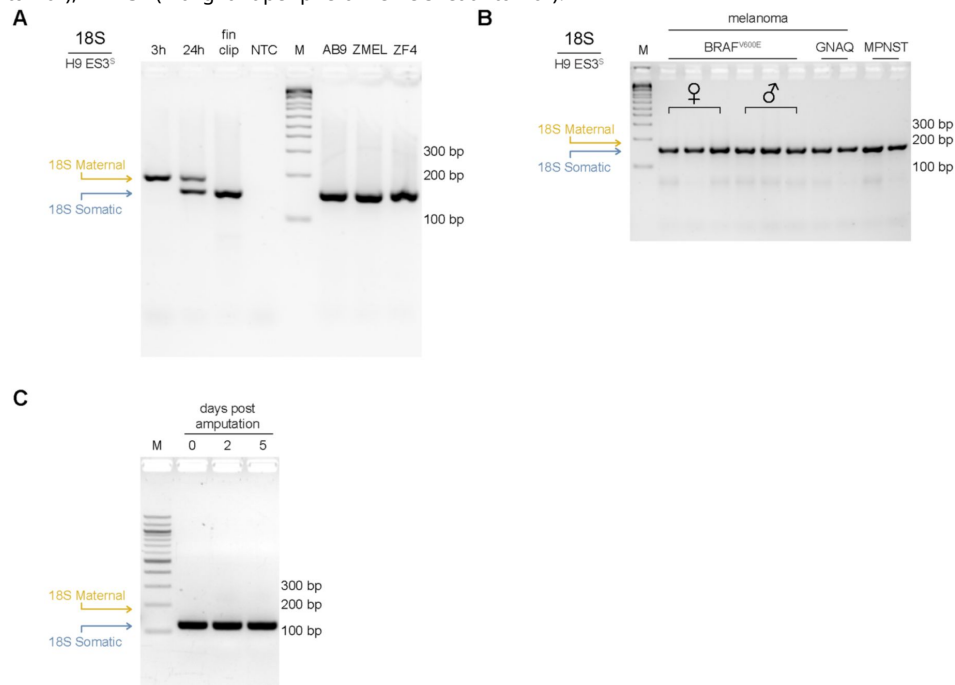
Expression of maternal (yellow) and somatic (blue) ITS1 sequences, indicative of de novo rRNA transcription, in **A**) whole embryos/larvae and in **B**) FACS-sorted PGCs. Public dataset PRJNA597223 (Redl et al. 2021 [DOI](#)) was obtained from the Sequence Read Archive at NCBI for analysis.

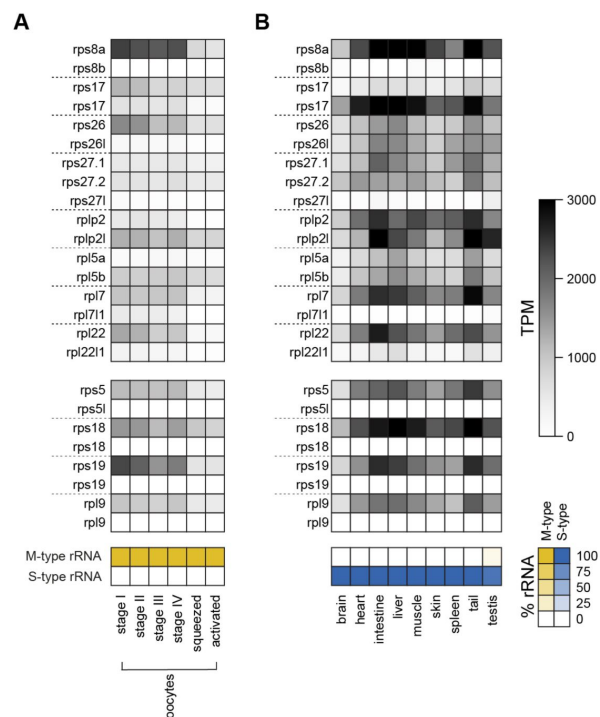


Supplementary Figure S4

Detecting rRNA variants in cell lines, tumors, and regenerating fins

A) Gel electrophoretic analysis of PCR-based fragment length polymorphism (FLP) 18S rRNA variant detection from the indicated cell lines. Diagnostic PCR lengths for maternal and somatic 18S rRNAs are indicated. Positive control reactions using 3 hpf, 24 hpf, and adult fin clip cDNAs are included to compare maternal-only, maternal and somatic, or somatic-only band patterns, respectively. **B**) Gel electrophoretic analysis of 18S rRNA variant detection from the indicated excised tumors. See Materials and Methods for a complete description of each cell line and tumor. **C**) Gel electrophoretic analysis of the 18S rRNA FLP assay using cDNA made from regenerating fin blastema excised at the indicated times post initial amputation. NTC, no template control. AB9 (fibroblast cells), ZMEL (melanoma cells), ZF4 (fibroblast cells), BRAF (melanoma tumor), GNAQ (melanoma tumor), MPNST (malignant peripheral nerve sheath tumor).

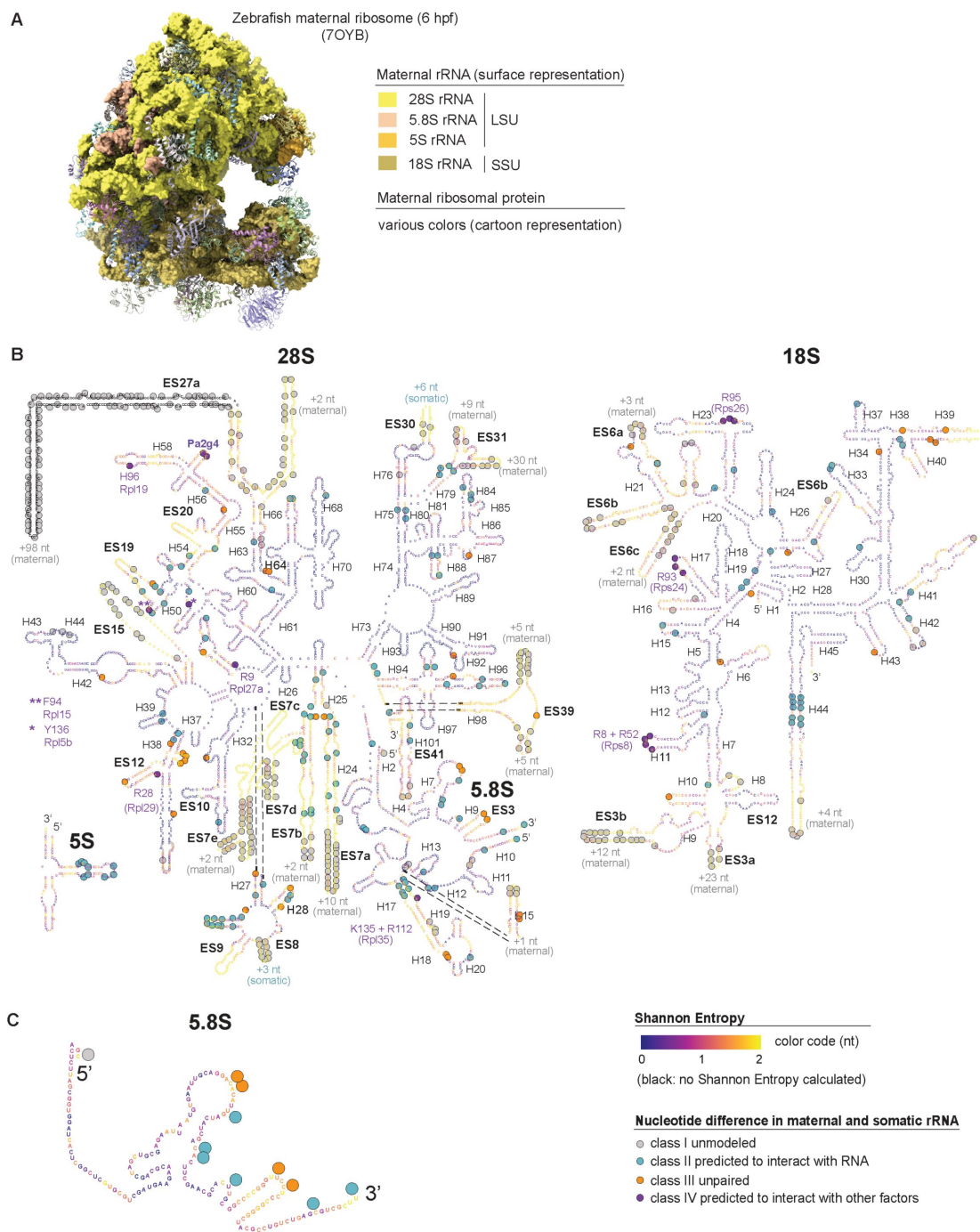




Supplementary Figure S5

Expression of alternative ribosomal core protein mRNAs in zebrafish oogenesis and adult tissues

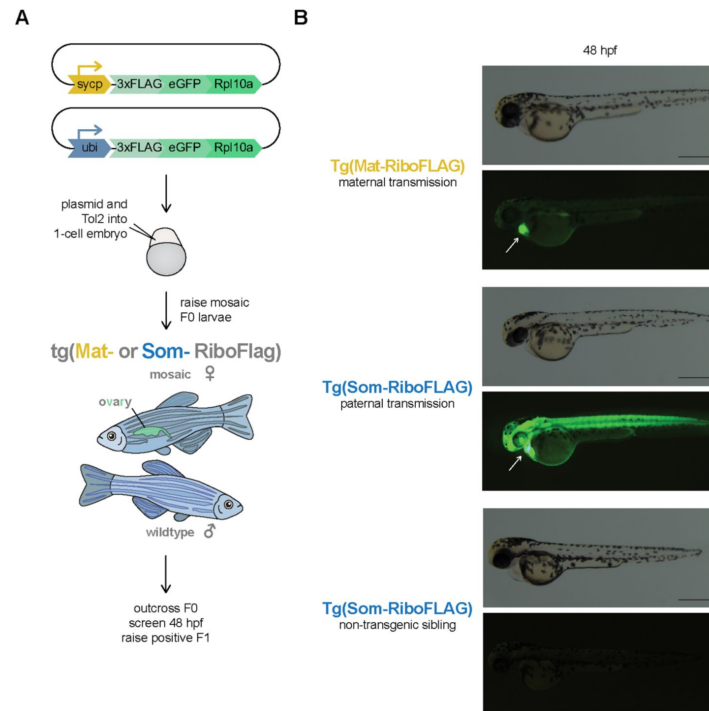
Heatmap of mRNA expression of paralogs and alternative isoforms encoding ribosomal protein variants assayed **A**) over progressive stages of oogenesis and **B**) across adult tissues and organs. The relative levels of maternal (yellow) or somatic (blue) rRNA variants at these developmental stages is indicated in the scheme below each heat map.



Supplementary Figure S6

Sites that differ between maternal and somatic rRNAs in zebrafish are enriched in evolutionarily less conserved regions

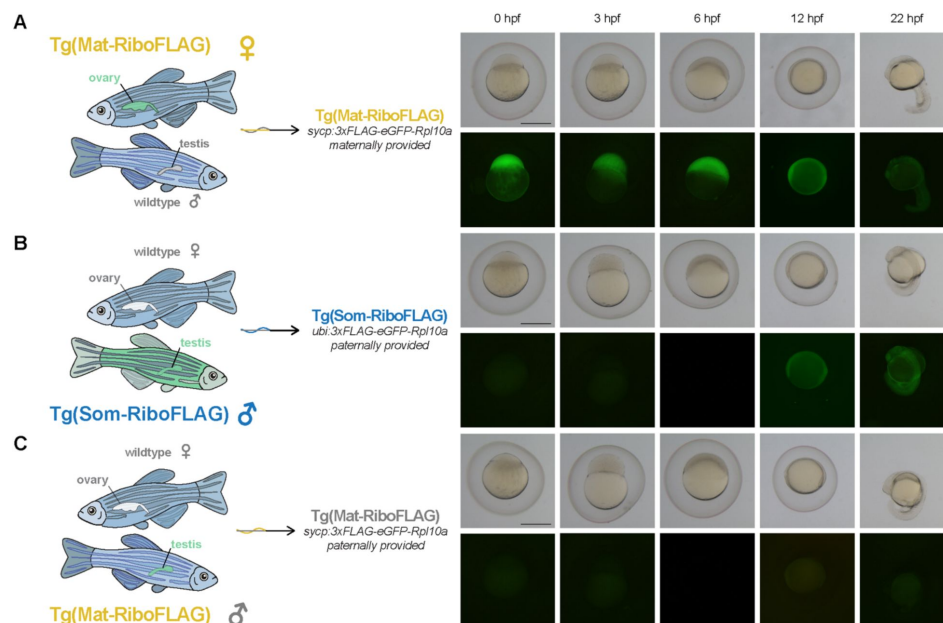
A) Map of the maternal zebrafish ribosome isolated from 6 hpf embryos (PDB: 7OYB; Leesch and Lorenzo-Orts et al., 2023). Maternal rRNA sequences are shown in surface representation in shades of yellow; ribosomal proteins are shown in cartoon representation in various colors. **B)** Positions of sequence differences in zebrafish maternal and somatic rRNA variants mapped onto rRNA secondary structure predictions of human 28S, 18S, 5.8S and 5S rRNAs, color-coded for site-specific Shannon Entropy values, which were downloaded from Ribovision2 (<https://ribovision2.chemistry.gatech.edu/>; Bernier et al., 2014). A higher magnification view of the 5.8S rRNA is shown in C), highlighting the co-occurrence of evolutionarily variable nucleotides (high Shannon Entropy values) with sites that differ between zebrafish maternal and somatic rRNAs.



Supplementary Figure S7

Generation of Tg(Mat-RiboFLAG) and Tg(Som-RiboFLAG) lines

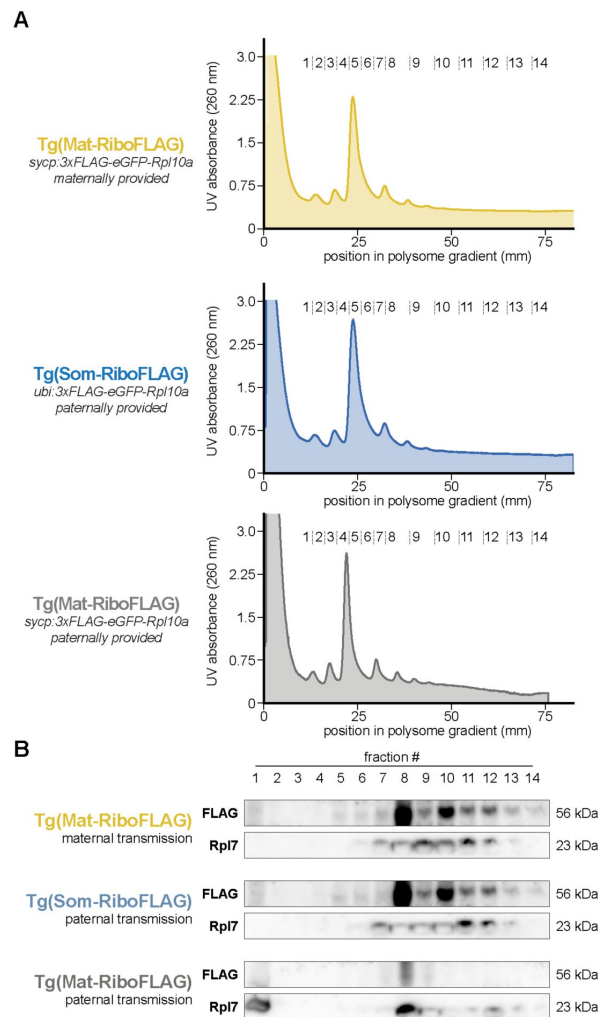
A) Cartoon depicting the generation of two transgenic lines containing either FLAG-tagged maternal or FLAG-tagged somatic ribosomes (see Materials and Methods for details). Mosaic F0 were raised to adulthood and transmission of either transgene via the germline was screened for in **B)** 48 hpf embryos using a green heart marker (*cmhc2* promoter driving eGFP) also encoded on the plasmid (Kwan et al. 2007 [\[1\]](#)) as indicated by the white arrow. As expected, only half of the progeny derived from a Tg(Som-RiboFLAG) male and a wildtype female express the transgene. Scale bars indicate 500 μ m. eGFP, enhanced green fluorescent protein. Tg, transgene.



Supplementary Figure S8

Transgenic expression 3xFLAG-eGFP-Rpl10a

Scheme summarizing the generation of embryos with FLAG-tagged ribosomes. Brightfield and fluorescent images of developing zebrafish embryos from either **A**) maternally-provided Tg(Mat-RiboFLAG), **B**) paternally-provided Tg(Som-RiboFLAG), or **C**) paternally-provided Tg(Mat-RiboFLAG) parents at the indicated hours post-fertilization. Embryos depicted in (A) contain maternally deposited 3xFLAG-eGFP-Rpl10a at 0 hpf while embryos depicted in (B) begin expression of 3xFLAG-eGFP-Rpl10a after 6 hpf. See **Supplementary Fig. S9** for expression at 48 hpf. As expected, progeny derived from paternally-provided Tg(Mat-RiboFLAG) show no transgene expression. Scale bars indicate 500 μ m.



Supplementary Figure S9

Incorporation of Tg(3xFLAG-Rpl10a) into translating ribosomes

A) Representative polysome profiles with continuous A260 reading from 24 hpf embryos. Position in the gradient and collected fractions are indicated. **B)** Western blots of polysome gradient fractions containing lysate from 24 hpf embryos with either maternally-provided Tg(Mat-RiboFLAG) or paternally-inherited Tg(Som-RiboFLAG) detecting the expression of transgenic FLAG-Rpl10a, and a negative control Western blot of polysome gradient fractions containing lysate from 24 hpf embryos with paternally-provided Tg(Mat-RiboFLAG) lacking the expression of transgenic FLAG-Rpl10a. kDa, kilodalton.

Supplemental Tables

Author Contributions

A.N.S.	conceptualization, data curation, formal analysis, investigation, methodology (in addition to general parts, hybrid ribosome and germline experiments), writing
F.L.	conceptualization, data curation, formal analysis, investigation, methodology (in addition to general parts, proteomics sample preparation and analysis), writing
L.L.-O.	data curation, formal analysis, investigation, writing
L.G.	formal analysis, investigation, methodology, validation (structural analysis)
M.N.	investigation, methodology (RNA-Seq analysis)
D.H.	data curation, formal analysis, supervision, validation
E.C.	conceptualization, project administration, supervision, funding acquisition, writing
A.P.	conceptualization, project administration, supervision, investigation, formal analysis, funding acquisition, writing

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gene name	Signal Area (5 dpf)			Signal Area (6 hpf)			log2fc [5d/6h]	p-value [5d/6h]
	rep I	rep II	rep III	rep I	rep II	rep III		
si:dkey-7j14.6	7.43E+04	5.32E+04	4.29E+04	2.37E+08	1.06E+08	3.63E+08	-11.88	1.46E-06
rmasel3	7.43E+04	5.32E+04	4.29E+04	3.66E+08	5.93E+07	1.46E+08	-11.37	7.21E-06
retsatl	7.43E+04	5.32E+04	4.29E+04	4.22E+07	9.07E+07	4.58E+07	-9.98	1.45E-06
gyg1a	7.43E+04	3.96E+05	4.29E+04	2.22E+08	2.06E+08	1.13E+06	-8.43	1.08E-02
slc43a1a	7.43E+04	5.32E+04	1.02E+05	2.07E+07	1.16E+07	2.69E+07	-7.98	6.32E-06
vldlr	7.43E+04	5.32E+04	4.29E+04	1.21E+07	3.78E+06	1.51E+07	-7.32	3.96E-05
si:ch73-189n23.1	7.43E+04	9.52E+06	2.94E+07	1.86E+08	1.41E+09	2.95E+08	-7.28	2.25E-02
rmasel2	1.85E+06	1.51E+06	6.88E+06	3.80E+08	2.40E+08	1.45E+08	-6.46	1.67E-04
rps17	3.26E+06	6.99E+07	9.77E+07	2.20E+09	2.49E+09	2.19E+09	-6.34	3.69E-03
lamp2	7.43E+04	5.32E+04	4.29E+04	1.62E+07	3.77E+06	1.13E+06	-6.21	9.72E-04
lamp1b	7.43E+04	5.32E+04	4.29E+04	8.55E+06	5.57E+05	1.12E+07	-6.09	2.79E-03
neb	4.81E+05	1.06E+04	3.15E+04	5.07E+06	6.98E+06	1.13E+06	-5.97	9.35E-03
ELAPOR2	7.43E+04	5.32E+04	4.29E+04	7.46E+06	1.57E+06	3.51E+06	-5.96	1.43E-04
hsp90b1	7.43E+04	3.79E+04	4.29E+04	2.32E+06	9.23E+06	1.13E+06	-5.87	5.60E-04
mast1a	7.43E+04	1.76E+06	3.58E+04	9.98E+06	9.81E+06	9.29E+06	-5.86	8.13E-03
tspan36	4.91E+05	4.61E+05	4.29E+04	1.69E+07	1.43E+07	5.96E+06	-5.72	2.24E-03
si:ch211-226h8.14	5.63E+05	5.32E+04	9.73E+05	1.50E+07	2.17E+08	1.13E+06	-5.65	4.04E-02
pdia4	7.43E+04	3.03E+05	4.29E+04	5.34E+06	1.11E+07	1.13E+06	-5.36	3.47E-03
tspan3a	3.37E+06	1.84E+06	4.29E+04	2.37E+07	1.61E+07	3.48E+07	-5.20	2.33E-02
itm2ba	7.43E+04	5.32E+04	4.29E+04	5.75E+06	3.17E+05	2.06E+06	-4.81	5.09E-03
tp1e	7.43E+04	5.32E+04	4.29E+04	2.73E+06	5.57E+05	2.29E+06	-4.78	6.96E-04
zgc:171717	4.88E+06	1.05E+07	4.67E+07	2.83E+08	7.10E+08	2.09E+08	-4.70	3.31E-03
tspan7b	7.43E+04	6.47E+05	2.56E+05	5.98E+06	3.24E+06	9.73E+06	-4.63	2.60E-03
naalad2	1.37E+05	5.32E+04	4.29E+04	2.18E+06	5.54E+05	3.80E+06	-4.61	2.24E-03
sptbn1	1.52E+05	3.46E+04	3.64E+04	4.23E+05	5.57E+05	5.81E+06	-4.27	1.32E-02
myh9b	1.41E+05	4.38E+03	7.62E+04	4.23E+05	5.57E+05	1.13E+06	-4.15	2.48E-02
myom1a	7.43E+04	9.96E+04	7.12E+03	4.23E+05	5.57E+05	1.13E+06	-4.10	1.13E-02
znf1157	5.46E+04	2.68E+04	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-4.02	4.35E-04
pon3.2	7.43E+04	3.08E+05	4.29E+04	4.60E+05	5.76E+06	1.13E+06	-3.86	1.85E-02
ryr3	3.42E+04	6.01E+04	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-3.85	4.37E-04
prdx4	7.43E+04	7.37E+04	4.29E+04	3.96E+05	1.52E+06	1.13E+06	-3.84	1.14E-03
krt15	7.43E+04	2.88E+04	4.40E+04	4.23E+05	5.57E+05	1.13E+06	-3.82	8.02E-04
jupa	3.59E+04	3.13E+05	4.29E+04	4.23E+05	2.57E+06	1.13E+06	-3.77	1.49E-02
tm9sf2	7.43E+04	5.32E+04	4.29E+04	1.09E+06	6.76E+05	4.87E+05	-3.68	3.42E-04
zgc:174164	7.43E+04	5.32E+04	4.29E+04	5.04E+05	5.09E+05	1.13E+06	-3.58	5.18E-04
mrpl44	7.43E+04	3.04E+04	7.41E+04	4.23E+05	5.57E+05	1.13E+06	-3.55	1.34E-03
myha	7.43E+04	8.94E+04	3.29E+04	4.23E+05	5.57E+05	1.13E+06	-3.42	1.70E-03
calr	7.43E+04	1.94E+05	4.29E+04	4.32E+05	1.44E+06	1.13E+06	-3.39	5.18E-03
bms1	3.62E+04	1.61E+05	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-3.35	4.79E-03
zgc:113102	2.95E+05	2.38E+04	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-3.26	2.13E-02
ddx27	7.43E+04	1.91E+05	2.40E+04	4.23E+05	5.57E+05	1.13E+06	-3.20	1.14E-02
actn3b	7.43E+04	7.60E+04	6.19E+04	4.23E+05	5.57E+05	1.13E+06	-3.19	8.48E-04
emg1	7.43E+04	1.27E+05	4.39E+04	4.23E+05	5.57E+05	1.13E+06	-3.11	2.70E-03
nol11	7.43E+04	2.83E+05	2.39E+04	4.23E+05	5.57E+05	1.13E+06	-3.02	2.40E-02
exosc10	5.28E+04	1.98E+05	4.85E+04	4.23E+05	5.57E+05	1.13E+06	-3.01	7.15E-03

Supplementary Table S1

Proteins differentially associated with maternal and somatic zebrafish ribosomes.

Proteins significantly enriched or depleted in ribosomes isolated from 6 hpf embryos versus 5 dpf larvae (permutation-based FDR <0.05).

vtg4	1.74E+06	5.83E+06	1.12E+07	7.33E+07	5.51E+07	1.39E+07	-2.98	2.26E-02
BX548076.3	7.43E+04	7.93E+05	1.15E+06	4.23E+05	6.71E+07	1.13E+06	-2.96	2.21E-01
rrp12	8.01E+04	2.70E+05	2.59E+04	4.23E+05	5.57E+05	1.13E+06	-2.96	2.21E-02
srpk1a	7.43E+04	3.18E+04	2.41E+05	4.23E+05	5.57E+05	1.13E+06	-2.96	1.49E-02
si:dkey-159f12.2	1.01E+06	5.32E+04	1.06E+06	4.23E+05	5.46E+07	1.13E+06	-2.95	2.25E-01
rpl37	4.89E+07	3.34E+08	2.49E+07	5.39E+08	7.02E+08	4.74E+08	-2.93	2.85E-02
eif4a1a	7.43E+04	4.24E+05	2.20E+04	4.23E+05	5.57E+05	1.13E+06	-2.86	4.82E-02
mtldha	7.43E+04	4.65E+04	2.02E+05	4.23E+05	5.57E+05	1.13E+06	-2.86	8.03E-03
utp4	7.43E+04	1.64E+05	5.75E+04	4.23E+05	5.57E+05	1.13E+06	-2.86	4.23E-03
si:dkey-65b12.6	7.43E+04	1.40E+05	6.76E+04	4.23E+05	5.57E+05	1.13E+06	-2.86	2.71E-03
aqr	7.43E+04	2.61E+05	3.70E+04	4.23E+05	5.57E+05	1.13E+06	-2.85	1.63E-02
ywhabb	7.43E+04	2.53E+05	4.11E+04	4.23E+05	5.57E+05	1.13E+06	-2.81	1.45E-02
ddx54	3.62E+04	1.26E+05	1.72E+05	4.23E+05	5.57E+05	1.13E+06	-2.80	1.10E-02
lsg1	6.40E+04	9.69E+04	1.31E+05	4.23E+05	5.57E+05	1.13E+06	-2.79	2.76E-03
utp20	1.15E+05	1.80E+05	3.96E+04	4.23E+05	5.57E+05	1.13E+06	-2.78	9.79E-03
ddx52	7.43E+04	2.55E+05	5.54E+04	4.23E+05	5.57E+05	1.13E+06	-2.66	1.30E-02
mybphb	7.43E+04	9.33E+04	1.71E+05	4.23E+05	5.57E+05	1.13E+06	-2.60	4.63E-03
cct8	7.43E+04	2.54E+05	7.50E+04	4.23E+05	5.57E+05	1.13E+06	-2.52	1.22E-02
prrc2c	7.43E+04	8.47E+04	2.26E+05	4.23E+05	5.57E+05	1.13E+06	-2.52	9.04E-03
dhx9	7.43E+04	1.24E+05	1.54E+05	4.23E+05	5.57E+05	1.13E+06	-2.52	4.63E-03
tbl3	7.13E+04	4.69E+05	4.32E+04	4.23E+05	5.57E+05	1.13E+06	-2.51	4.83E-02
rbfox2	1.65E+05	2.12E+05	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-2.49	1.95E-02
adar	7.43E+04	1.33E+05	1.66E+05	4.23E+05	5.57E+05	1.13E+06	-2.45	5.85E-03
vtg1	1.70E+07	7.30E+07	7.78E+07	3.72E+08	3.36E+08	1.26E+08	-2.45	2.42E-02
kpnb3	7.43E+04	1.48E+05	1.61E+05	4.23E+05	5.57E+05	1.13E+06	-2.41	6.40E-03
heatr1	6.26E+04	2.70E+05	1.05E+05	4.23E+05	5.57E+05	1.13E+06	-2.41	1.60E-02
ywhae1	7.43E+04	4.43E+05	5.75E+04	4.23E+05	5.57E+05	1.13E+06	-2.38	4.27E-02
wdr75	7.43E+04	1.98E+05	1.30E+05	4.23E+05	5.57E+05	1.13E+06	-2.37	8.36E-03
eef2l2	7.43E+04	6.93E+04	3.78E+05	4.23E+05	5.57E+05	1.13E+06	-2.37	3.07E-02
ddx18	7.43E+04	2.52E+05	1.10E+05	4.23E+05	5.57E+05	1.13E+06	-2.34	1.29E-02
elavl1b	3.16E+05	1.56E+05	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-2.33	3.69E-02
wdr12	7.43E+04	5.67E+05	5.18E+04	4.23E+05	5.57E+05	1.13E+06	-2.31	6.68E-02
mrpl15	1.68E+05	3.04E+05	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-2.31	3.71E-02
utp6	7.43E+04	2.04E+05	1.62E+05	4.23E+05	5.57E+05	1.13E+06	-2.25	1.16E-02
ufi1	7.43E+04	3.85E+05	9.04E+04	4.23E+05	5.57E+05	1.13E+06	-2.23	3.29E-02
crnk1l	7.43E+04	4.22E+05	8.31E+04	4.23E+05	5.57E+05	1.13E+06	-2.23	3.94E-02
bysl	7.43E+04	7.29E+04	4.92E+05	4.23E+05	5.57E+05	1.13E+06	-2.22	5.25E-02
luc7l	1.43E+06	4.52E+04	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-2.20	1.90E-01
ywhaz	1.86E+05	3.60E+05	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-2.18	5.44E-02
rpl29	4.08E+08	1.51E+08	2.27E+08	1.02E+09	9.74E+08	1.27E+09	-2.17	5.55E-03
hspb1	7.43E+04	6.11E+05	6.61E+04	4.23E+05	5.57E+05	1.13E+06	-2.16	7.64E-02
eif2s1a	7.43E+04	1.41E+05	2.92E+05	4.23E+05	5.57E+05	1.13E+06	-2.15	2.17E-02
ktn1	1.14E+05	2.20E+05	1.31E+05	4.23E+05	5.57E+05	1.13E+06	-2.12	9.36E-03
RPL37A	8.70E+08	2.05E+09	3.99E+09	7.94E+09	8.42E+09	8.06E+09	-2.08	1.81E-02
hdlbpa	7.43E+04	1.94E+05	2.47E+05	4.23E+05	5.57E+05	1.13E+06	-2.08	2.17E-02
rcl1	7.43E+04	4.08E+05	1.21E+05	4.23E+05	5.57E+05	1.13E+06	-2.06	4.10E-02
pkhd1l1	7.43E+04	3.29E+05	1.52E+05	4.23E+05	5.57E+05	1.13E+06	-2.05	3.01E-02
cdk5rap3	7.04E+04	3.35E+05	1.76E+05	4.23E+05	5.57E+05	1.13E+06	-2.00	3.63E-02
plrg1	7.43E+04	4.39E+05	1.27E+05	4.23E+05	5.57E+05	1.13E+06	-2.00	4.89E-02
metap1	7.43E+04	1.87E+05	3.14E+05	4.23E+05	5.57E+05	1.13E+06	-1.98	3.32E-02
eif3m	7.43E+04	1.88E+05	3.22E+05	4.23E+05	5.57E+05	1.13E+06	-1.96	3.53E-02

Supplementary Table S1 (continued)

flot2a	7.43E+04	4.26E+05	1.44E+05	4.23E+05	5.57E+05	1.13E+06	-1.96	4.94E-02
nme2b.2	7.43E+04	1.42E+06	4.38E+04	4.23E+05	5.57E+05	1.13E+06	-1.95	2.14E-01
ttn.2	1.77E+05	2.33E+05	1.15E+05	4.23E+05	5.57E+05	1.13E+06	-1.94	1.40E-02
abce1	7.43E+04	1.85E+05	3.61E+05	4.23E+05	5.57E+05	1.13E+06	-1.92	4.34E-02
si:ch211-198a12.6	1.05E+05	1.11E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-1.91	1.85E-01
atp2a2a	1.11E+05	1.11E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-1.89	1.88E-01
rps27a	2.13E+08	3.20E+08	6.31E+08	1.34E+09	1.31E+09	1.19E+09	-1.87	1.22E-02
krt17	7.43E+04	3.83E+05	2.16E+05	4.23E+05	5.57E+05	1.13E+06	-1.81	5.69E-02
dnaja2b	7.43E+04	6.20E+05	1.59E+05	4.23E+05	5.57E+05	1.13E+06	-1.73	1.04E-01
ltv1	7.43E+04	2.89E+05	3.45E+05	4.23E+05	5.57E+05	1.13E+06	-1.72	6.75E-02
plecb	7.95E+05	2.69E+05	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-1.62	2.07E-01
tuba8l3	7.43E+04	1.65E+06	9.26E+04	4.23E+05	5.57E+05	1.13E+06	-1.52	2.88E-01
rplp1	2.75E+08	3.16E+08	3.36E+08	7.28E+08	9.78E+08	9.66E+08	-1.52	7.44E-03
ldhba	7.43E+04	1.17E+06	1.99E+05	4.23E+05	5.57E+05	1.13E+06	-1.32	2.74E-01
pbdcl	7.43E+04	1.12E+06	2.09E+05	4.23E+05	5.57E+05	1.13E+06	-1.32	2.68E-01
ufm1	7.43E+04	1.87E+06	1.26E+05	4.23E+05	5.57E+05	1.13E+06	-1.31	3.54E-01
fbxl6	3.30E+05	4.94E+05	1.17E+05	4.23E+05	5.57E+05	1.13E+06	-1.27	1.29E-01
arglu1a	7.43E+04	5.24E+05	6.13E+05	4.23E+05	5.57E+05	1.13E+06	-1.16	2.72E-01
rps27l	3.62E+08	2.08E+08	2.38E+08	5.59E+08	5.92E+08	5.90E+08	-1.15	3.11E-02
cyt1l	7.43E+04	3.44E+05	1.03E+06	4.23E+05	5.57E+05	1.13E+06	-1.12	3.27E-01
naa40	7.43E+04	2.37E+05	1.52E+06	4.23E+05	5.57E+05	1.13E+06	-1.11	3.81E-01
exosc4	7.43E+04	1.06E+06	3.78E+05	4.23E+05	5.57E+05	1.13E+06	-1.06	3.57E-01
npm1a	7.52E+05	4.64E+04	9.01E+05	4.23E+05	5.57E+05	1.13E+06	-1.03	4.46E-01
rps12	3.46E+09	3.58E+09	4.58E+09	7.69E+09	7.87E+09	7.44E+09	-1.00	3.64E-02
si:ch211-106j21.4	8.00E+05	6.38E+04	7.12E+05	4.23E+05	5.57E+05	1.13E+06	-0.96	4.22E-01
sf3b4	7.43E+04	1.41E+06	3.58E+05	4.23E+05	5.57E+05	1.13E+06	-0.94	4.39E-01
h2ax	3.69E+06	8.92E+06	4.29E+04	4.23E+05	1.81E+07	1.13E+06	-0.87	7.32E-01
hnmpul1	1.14E+06	1.02E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-0.81	5.83E-01
cryba2a	9.85E+05	1.24E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-0.78	5.97E-01
srp9	7.43E+04	7.24E+05	1.01E+06	4.23E+05	5.57E+05	1.13E+06	-0.76	5.16E-01
si:ch73-366l1.5	8.74E+05	1.71E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-0.69	6.54E-01
cryba1b	1.69E+05	1.33E+06	3.04E+05	4.23E+05	5.57E+05	1.13E+06	-0.65	4.92E-01
mdka	7.43E+04	1.61E+06	6.19E+05	4.23E+05	5.57E+05	1.13E+06	-0.62	6.26E-01
imp3	7.43E+04	1.56E+06	6.82E+05	4.23E+05	5.57E+05	1.13E+06	-0.58	6.45E-01
cirbpb	7.43E+04	6.81E+05	1.60E+06	4.23E+05	5.57E+05	1.13E+06	-0.57	6.53E-01
srsf6a	7.43E+04	3.69E+06	4.06E+05	4.23E+05	5.57E+05	1.13E+06	-0.42	7.81E-01
si:dkey-108k21.21	7.43E+04	1.88E+06	9.79E+05	4.23E+05	5.57E+05	1.13E+06	-0.32	8.11E-01
snrpd3	1.54E+06	2.12E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	-0.31	8.52E-01
si:ch211-76m11.8	2.28E+05	7.47E+05	9.70E+05	4.23E+05	5.57E+05	1.13E+06	-0.23	7.63E-01
rbfox1	7.43E+04	1.06E+06	2.22E+06	4.23E+05	5.57E+05	1.13E+06	-0.20	8.85E-01
rpl9	7.43E+04	2.09E+06	1.22E+06	4.23E+05	5.57E+05	1.13E+06	-0.16	9.07E-01
chtopa	7.43E+04	3.64E+06	7.17E+05	4.23E+05	5.57E+05	1.13E+06	-0.15	9.19E-01
tnnt3b	1.94E+06	4.87E+05	2.22E+05	4.23E+05	5.57E+05	1.13E+06	-0.12	9.02E-01
cirbpa	7.43E+04	1.69E+06	1.81E+06	4.23E+05	5.57E+05	1.13E+06	-0.08	9.57E-01
srp19	7.43E+04	1.78E+06	2.01E+06	4.23E+05	5.57E+05	1.13E+06	0.00	9.98E-01
zgc:136908	1.59E+05	1.66E+06	1.19E+06	4.23E+05	5.57E+05	1.13E+06	0.08	9.40E-01
noi12	4.66E+05	1.42E+06	5.11E+05	4.23E+05	5.57E+05	1.13E+06	0.11	8.68E-01
mylpfb	3.39E+06	2.99E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	0.23	9.01E-01
ppiab	8.49E+05	2.15E+06	3.65E+05	4.23E+05	5.57E+05	1.13E+06	0.44	6.00E-01
mrrpl41	1.18E+06	2.32E+06	2.71E+05	4.23E+05	5.57E+05	1.13E+06	0.49	6.11E-01
flna	2.25E+06	6.77E+05	5.48E+05	4.23E+05	5.57E+05	1.13E+06	0.55	4.80E-01

Supplementary Table S1 (continued)

hnrnpa0l	7.43E+04	4.10E+06	2.80E+06	4.23E+05	5.57E+05	1.13E+06	0.56	7.42E-01
ccdc59	2.48E+06	5.74E+05	6.20E+05	4.23E+05	5.57E+05	1.13E+06	0.57	4.78E-01
purbb	1.27E+07	4.51E+05	1.77E+05	4.23E+05	5.57E+05	1.13E+06	0.64	7.09E-01
snrpg	7.43E+04	2.56E+06	6.22E+06	4.23E+05	5.57E+05	1.13E+06	0.72	6.89E-01
pfnd4	7.43E+04	4.59E+06	4.83E+06	4.23E+05	5.57E+05	1.13E+06	0.87	6.33E-01
eif1axa	1.94E+06	8.85E+05	9.97E+05	4.23E+05	5.57E+05	1.13E+06	0.89	1.75E-01
tmem67	7.54E+08	4.00E+08	3.92E+08	2.51E+08	2.54E+08	2.71E+08	0.93	8.02E-02
srsf3a	5.93E+05	1.61E+06	3.12E+06	4.23E+05	5.57E+05	1.13E+06	1.16	1.82E-01
snrpe	7.43E+04	8.61E+06	5.06E+06	4.23E+05	5.57E+05	1.13E+06	1.20	5.47E-01
tp53inp2	7.43E+04	1.89E+07	3.09E+06	4.23E+05	5.57E+05	1.13E+06	1.34	5.33E-01
rps27.1, rps27.2	2.60E+09	1.44E+09	1.28E+09	6.71E+08	6.13E+08	6.54E+08	1.39	2.10E-02
skiv2l	1.54E+06	1.77E+06	2.19E+06	4.23E+05	5.57E+05	1.13E+06	1.49	2.81E-02
zgc:153867	3.25E+06	1.15E+06	1.70E+06	4.23E+05	5.57E+05	1.13E+06	1.52	5.01E-02
gadd45gip1	4.20E+07	3.56E+06	4.29E+04	4.23E+05	5.57E+05	1.13E+06	1.53	5.60E-01
hmgb2b	1.26E+06	3.13E+06	1.66E+06	4.23E+05	5.57E+05	1.13E+06	1.54	4.25E-02
eef1a1l1	2.00E+06	4.97E+06	2.51E+06	1.37E+06	5.57E+05	1.13E+06	1.62	3.35E-02
eif3i	1.09E+06	1.97E+06	3.78E+06	4.23E+05	5.57E+05	1.13E+06	1.64	4.82E-02
crygn2	1.60E+06	3.20E+06	1.60E+06	4.23E+05	5.57E+05	1.13E+06	1.65	2.90E-02
rpl10	1.19E+08	8.41E+07	7.63E+07	2.18E+07	3.97E+07	2.61E+07	1.69	9.34E-03
hsp90ab1	1.06E+06	3.82E+06	2.74E+06	4.23E+05	5.57E+05	1.13E+06	1.79	4.01E-02
eif3ba	1.49E+06	2.66E+06	2.94E+06	4.23E+05	5.57E+05	1.13E+06	1.82	1.85E-02
srpk1b	1.76E+06	3.30E+06	2.13E+06	4.23E+05	5.57E+05	1.13E+06	1.84	1.59E-02
hmgb3a	1.18E+06	3.61E+06	3.59E+06	4.23E+05	5.57E+05	1.13E+06	1.95	2.85E-02
rt2a	1.27E+06	9.98E+06	1.35E+06	4.23E+05	5.57E+05	1.13E+06	2.00	8.32E-02
ilf3b	1.85E+06	1.76E+06	5.30E+06	4.23E+05	5.57E+05	1.13E+06	2.00	2.41E-02
mrpl12	2.13E+06	6.13E+06	1.47E+06	4.23E+05	5.57E+05	1.13E+06	2.06	2.97E-02
syncrip	3.65E+06	3.41E+06	1.74E+06	4.23E+05	5.57E+05	1.13E+06	2.12	1.09E-02
eif3ha	3.32E+06	2.85E+06	2.38E+06	4.23E+05	5.57E+05	1.13E+06	2.13	6.40E-03
mvp	7.31E+06	1.38E+07	4.58E+06	3.51E+06	1.12E+06	1.32E+06	2.16	1.97E-02
larplb	3.47E+06	3.03E+06	2.27E+06	4.23E+05	5.57E+05	1.13E+06	2.16	6.55E-03
ppan	1.36E+06	5.12E+06	3.63E+06	4.23E+05	5.57E+05	1.13E+06	2.19	2.02E-02
eif5a	2.99E+06	3.54E+06	2.54E+06	4.23E+05	5.57E+05	1.13E+06	2.22	5.35E-03
snd1	4.27E+06	5.84E+06	2.72E+06	9.19E+05	5.57E+05	1.13E+06	2.29	4.54E-03
eif3s10	2.94E+06	3.89E+06	2.76E+06	4.23E+05	5.57E+05	1.13E+06	2.30	4.69E-03
rs11d1	1.72E+06	3.85E+06	4.80E+06	4.23E+05	5.57E+05	1.13E+06	2.30	1.10E-02
eif4a3	2.22E+06	6.50E+06	2.54E+06	4.23E+05	5.57E+05	1.13E+06	2.37	1.10E-02
mvp	1.07E+09	1.10E+09	5.20E+08	3.32E+08	1.06E+08	1.21E+08	2.39	9.88E-03
naa15a	3.16E+06	2.46E+06	5.07E+06	4.23E+05	5.57E+05	1.13E+06	2.40	5.63E-03
nop2	1.39E+06	5.13E+06	5.60E+06	4.23E+05	5.57E+05	1.13E+06	2.41	1.81E-02
rnps1	3.83E+06	4.98E+06	2.28E+06	4.23E+05	5.57E+05	1.13E+06	2.45	5.59E-03
tubb5	3.86E+06	2.98E+06	3.79E+06	4.23E+05	5.57E+05	1.13E+06	2.45	3.27E-03
ckmb	3.26E+06	7.08E+06	1.96E+06	4.23E+05	5.57E+05	1.13E+06	2.47	1.11E-02
akap8l	2.57E+06	4.65E+06	3.91E+06	4.23E+05	5.57E+05	1.13E+06	2.48	4.13E-03
pfnd6	2.12E+06	5.27E+06	4.56E+06	4.23E+05	5.57E+05	1.13E+06	2.52	6.31E-03
rpl7l1	3.00E+06	6.24E+06	2.74E+06	4.23E+05	5.57E+05	1.13E+06	2.53	5.59E-03
eif3c	2.40E+06	3.46E+06	6.21E+06	4.23E+05	5.57E+05	1.13E+06	2.53	6.05E-03
si:dkey-183i3.5	1.52E+06	1.48E+07	2.33E+06	4.23E+05	5.57E+05	1.13E+06	2.54	4.23E-02
eif3s6ip	5.80E+06	4.18E+06	2.87E+06	4.23E+05	5.57E+05	1.13E+06	2.68	3.27E-03
tuba8l4	1.86E+06	7.58E+06	5.34E+06	4.23E+05	5.57E+05	1.13E+06	2.71	9.51E-03
srsf1a	4.21E+06	2.65E+06	6.80E+06	4.23E+05	5.57E+05	1.13E+06	2.72	4.26E-03
hnrnpaba	1.77E+06	6.29E+06	7.52E+06	4.23E+05	5.57E+05	1.13E+06	2.77	1.04E-02

Supplementary Table S1 (continued)

tuba8l	3.00E+06	9.41E+06	2.98E+06	4.23E+05	5.57E+05	1.13E+06	2.77	7.05E-03
krt91	1.45E+06	9.71E+06	6.71E+06	4.23E+05	5.57E+05	1.13E+06	2.82	1.78E-02
ebna1bp2	2.64E+07	1.16E+07	5.08E+06	6.91E+06	5.57E+05	1.13E+06	2.83	4.73E-02
zgc:163061	6.71E+06	1.15E+07	1.29E+06	4.23E+05	5.57E+05	1.13E+06	2.85	2.37E-02
zgc:56095	7.56E+07	5.00E+07	9.13E+07	8.43E+06	1.53E+07	6.60E+06	2.89	1.47E-03
ftbl	4.96E+06	6.09E+06	3.63E+06	4.23E+05	5.57E+05	1.13E+06	2.89	1.79E-03
znf622	9.58E+05	1.13E+07	1.03E+07	4.23E+05	5.57E+05	1.13E+06	2.90	3.90E-02
elf3ea	8.18E+06	2.48E+06	5.81E+06	4.23E+05	5.57E+05	1.13E+06	2.93	4.71E-03
ftsj3	5.23E+06	5.80E+06	3.89E+06	4.23E+05	5.57E+05	1.13E+06	2.93	1.51E-03
bxdc2	3.81E+06	1.00E+07	3.09E+06	4.23E+05	5.57E+05	1.13E+06	2.93	4.90E-03
btf3	6.56E+08	6.95E+08	1.24E+09	6.39E+07	4.12E+08	4.53E+07	2.96	2.00E-02
rrs1	2.26E+06	6.45E+06	8.90E+06	4.23E+05	5.57E+05	1.13E+06	2.97	6.07E-03
srsf7a	7.12E+06	3.45E+06	5.97E+06	4.23E+05	5.57E+05	1.13E+06	3.03	1.90E-03
icn2	7.84E+06	1.04E+07	2.36E+06	4.23E+05	5.57E+05	1.13E+06	3.16	5.73E-03
rpf2	3.91E+06	4.91E+06	1.02E+07	4.23E+05	5.57E+05	1.13E+06	3.17	2.23E-03
rsf24d1	3.69E+06	6.55E+06	8.26E+06	4.23E+05	5.57E+05	1.13E+06	3.18	1.68E-03
nsa2	4.99E+06	7.18E+06	6.52E+06	4.23E+05	5.57E+05	1.13E+06	3.26	8.52E-04
hbae1.3	7.18E+06	1.32E+07	2.67E+06	4.23E+05	5.57E+05	1.13E+06	3.30	5.05E-03
hnmpa1a	1.82E+06	7.60E+06	1.92E+07	4.23E+05	5.57E+05	1.13E+06	3.32	1.46E-02
zc3h15	3.30E+06	8.98E+06	9.27E+06	4.23E+05	5.57E+05	1.13E+06	3.34	2.31E-03
calm3a	7.34E+07	4.58E+07	2.63E+07	1.47E+07	5.13E+06	1.13E+06	3.34	1.83E-02
elf6	4.39E+06	1.75E+07	2.63E+07	4.17E+05	4.08E+06	1.13E+06	3.34	2.28E-02
apoa2	4.59E+06	1.49E+07	4.20E+06	4.23E+05	5.57E+05	1.13E+06	3.36	3.35E-03
si:dkey-12j5.1	1.23E+07	7.43E+06	3.68E+06	4.23E+05	5.57E+05	1.13E+06	3.43	2.14E-03
gnl3	5.30E+06	9.15E+06	7.23E+06	4.23E+05	5.57E+05	1.13E+06	3.45	7.55E-04
ifrd1	1.92E+07	5.08E+06	3.62E+06	4.23E+05	5.57E+05	1.13E+06	3.46	5.12E-03
naa15b	6.17E+06	5.43E+06	1.35E+07	4.23E+05	5.57E+05	1.13E+06	3.57	1.19E-03
igf2bp1	4.20E+06	1.17E+07	9.92E+06	4.23E+05	5.57E+05	1.13E+06	3.61	1.38E-03
ncl	2.24E+07	9.76E+06	2.45E+06	4.23E+05	5.57E+05	1.13E+06	3.66	8.01E-03
krt5	3.07E+06	1.49E+07	1.34E+07	4.23E+05	5.57E+05	1.13E+06	3.72	3.66E-03
tnnc2	1.96E+07	5.15E+06	6.31E+06	4.23E+05	5.57E+05	1.13E+06	3.74	2.07E-03
tnnt3b	2.41E+07	5.14E+06	5.28E+06	4.23E+05	5.57E+05	1.13E+06	3.75	3.53E-03
actb1	1.78E+06	1.43E+07	1.19E+07	1.73E+05	5.57E+05	1.13E+06	3.81	1.41E-02
srsf4	1.50E+07	8.32E+06	6.47E+06	4.23E+05	5.57E+05	1.13E+06	3.85	6.66E-04
lyar	2.51E+06	1.86E+07	2.15E+07	4.23E+05	5.57E+05	1.13E+06	3.96	7.09E-03
khdrbs1a	6.62E+06	1.86E+07	8.87E+06	4.23E+05	5.57E+05	1.13E+06	4.00	7.73E-04
snrpd2	1.79E+07	1.24E+07	5.15E+06	4.23E+05	5.57E+05	1.13E+06	4.02	1.09E-03
naa10	4.78E+06	1.31E+07	2.07E+07	4.23E+05	5.57E+05	1.13E+06	4.08	1.49E-03
apoa1a	4.09E+06	2.42E+07	1.44E+07	4.23E+05	5.57E+05	1.13E+06	4.13	2.47E-03
snrpd3l	9.46E+06	1.58E+07	9.71E+06	4.23E+05	5.57E+05	1.13E+06	4.14	3.04E-04
si:dkey-164f24.2	2.97E+07	1.21E+07	4.58E+06	4.23E+05	5.57E+05	1.13E+06	4.20	2.43E-03
si:ch211-113a14.11	1.87E+07	4.02E+07	2.20E+07	3.98E+06	5.57E+05	1.13E+06	4.23	2.43E-03
srsf3b	5.66E+06	1.33E+07	2.86E+07	4.23E+05	5.57E+05	1.13E+06	4.32	1.38E-03
si:dkey-108k21.10	2.27E+07	1.97E+07	5.67E+06	4.23E+05	5.57E+05	1.13E+06	4.40	1.06E-03
srsf5b	1.05E+07	1.75E+07	1.54E+07	4.23E+05	5.57E+05	1.13E+06	4.46	1.91E-04
myhz1.1	2.14E+07	1.59E+07	9.16E+06	4.23E+05	5.57E+05	1.13E+06	4.50	2.95E-04
si:ch73-368j24.11	1.25E+07	2.23E+07	1.14E+07	4.23E+05	5.57E+05	1.13E+06	4.51	2.38E-04
serbp1a	1.10E+09	6.13E+08	9.22E+08	8.03E+07	2.78E+07	2.27E+07	4.53	4.17E-04
gtbbp4	1.52E+07	1.83E+07	1.18E+07	4.23E+05	5.57E+05	1.13E+06	4.53	1.59E-04
atp5f1b	1.71E+07	4.54E+07	4.33E+06	4.23E+05	5.57E+05	1.13E+06	4.54	3.59E-03
LO017835.1	4.14E+06	2.27E+07	3.70E+07	4.23E+05	5.57E+05	1.13E+06	4.56	3.21E-03

Supplementary Table S1 (continued)

Supplementary Table S1 (continued)

myl1	7.60E+06	2.84E+07	1.80E+07	4.23E+05	5.57E+05	1.13E+06	4.61	6.03E-04
fkbp1aa	1.42E+07	1.88E+07	1.88E+07	4.23E+05	5.57E+05	1.13E+06	4.73	1.13E-04
si:ch211-113a14.12	2.07E+07	2.41E+07	1.69E+07	4.23E+05	5.57E+05	1.13E+06	4.98	8.75E-05
mylz3	2.20E+07	1.81E+07	2.82E+07	4.23E+05	5.57E+05	1.13E+06	5.12	8.20E-05
mrto4	1.39E+07	3.08E+07	3.52E+07	4.23E+05	5.57E+05	1.13E+06	5.26	1.63E-04
drg1	5.99E+07	2.39E+07	1.10E+07	4.23E+05	5.57E+05	1.13E+06	5.28	5.66E-04
srsf6b	1.22E+07	3.77E+07	4.34E+07	4.23E+05	5.57E+05	1.13E+06	5.40	2.90E-04
srsf5a	1.92E+07	3.47E+07	3.14E+07	4.23E+05	5.57E+05	1.13E+06	5.42	7.64E-05
actc1b	2.90E+08	1.16E+08	1.05E+08	4.60E+06	7.00E+06	1.13E+06	5.52	7.17E-04
si:ch211-133n4.4	4.97E+06	6.19E+07	1.05E+08	4.23E+05	5.57E+05	1.13E+06	5.63	4.30E-03
tpp2	3.02E+07	4.63E+07	2.50E+07	4.23E+05	5.57E+05	1.13E+06	5.67	5.93E-05
mylpfa	3.79E+07	3.06E+07	3.12E+07	4.23E+05	5.57E+05	1.13E+06	5.68	3.86E-05
cyt1	1.84E+07	6.86E+07	5.18E+07	4.23E+05	5.57E+05	1.13E+06	5.97	1.67E-04
pvalb2	3.41E+07	1.18E+08	2.05E+07	4.23E+05	5.57E+05	1.13E+06	6.08	3.27E-04
gapdh	5.44E+06	4.43E+08	4.18E+08	4.23E+05	6.72E+06	1.13E+06	6.09	2.55E-02
fthl27	9.02E+07	3.00E+07	4.00E+07	4.23E+05	5.57E+05	1.13E+06	6.21	8.49E-05
tuba1a	3.93E+07	8.84E+07	3.37E+07	4.23E+05	5.57E+05	1.13E+06	6.25	6.78E-05
hspa8	5.78E+07	9.34E+07	7.87E+07	4.23E+05	1.86E+06	1.13E+06	6.29	9.15E-05
zgc:55461	3.07E+07	1.03E+08	4.07E+07	4.23E+05	5.57E+05	1.13E+06	6.29	9.95E-05
tpma	5.27E+08	3.43E+07	3.61E+07	4.23E+05	5.57E+05	1.13E+06	7.07	1.19E-03
llph	8.31E+07	9.11E+07	9.01E+07	4.23E+05	5.57E+05	1.13E+06	7.10	1.05E-05
serbp1b	1.66E+08	7.71E+07	1.23E+08	4.23E+05	5.57E+05	1.13E+06	7.50	1.56E-05
atp2a1	2.08E+08	2.56E+08	5.44E+07	4.23E+05	5.57E+05	1.13E+06	7.79	6.82E-05
CABZ01093502.2	1.20E+09	8.84E+07	8.44E+07	4.23E+05	5.57E+05	1.13E+06	8.33	4.53E-04
rpl22l1	4.75E+08	9.10E+08	6.66E+08	4.23E+05	1.26E+07	1.13E+06	8.50	6.68E-04
zdhhc20b	4.69E+08	2.37E+08	2.48E+08	4.23E+05	5.57E+05	1.13E+06	8.87	5.94E-06
rpl5a	9.11E+08	7.62E+08	6.04E+08	4.23E+05	5.57E+05	1.13E+06	10.18	1.68E-06

Supplementary Table S3

Primer sequences and PCR conditions used to detect maternal and somatic rRNA variants by PCR-based fragment length polymorphism (FLP) and RT-qPCR.

Primer	Sequence	PCR	Products	Source
18S_ES3S_FLP_F	CTAATACATGCCAACGAGCGCC	NEB Taq 62 °C	200 bp from Mat18S 170 bp from Som18S	MIT IMP
18S_ES3S_FLP_R	CCGAGGTTATCTAGAGTCACCAAAAGC			
18S_Mat_qPCR_F	CGTGGGCGGTGGAGAGG	KAPA SYBR 68 °C	169 bp from Mat18S	MIT
18S_Mat_qPCR_R	CCATGGTAGGCGACGGACC			
18S_Som_qPCR_F	GCGGCGTGGGCTCCCCCTTCG	KAPA SYBR 68 °C	158 bp from Som18S	MIT
18S_Som_qPCR_R	CCATGGTAGGCGCCTAAAG			
28S_ES31L_FLP_F1	[M13]GGTGAAATACCACTACTCTTATCG	NEBNext Taq 68 °C	254* bp from Mat28S 210* bp from Som28S	MIT
28S_ES31L_FLP_R1	[PIG]GTTACCGTTTGACAGGTGTAC			
28S_ES31L_FLP_F2	CTCTTATCGTTTCTCACTTACCCG	1.3x Q5 + 1M betaine 70 °C	196 bp from Mat28S 152 bp from Som28S	IMP
28S_ES31L_FLP_R2	TACCGCCCCAGTCAAACCTCC			
28S_Mat_qPCR_F	AGGTGCCCTGACCCCGTTCCCGAGCCG	KAPA SYBR 68 °C	155 bp from Mat28S	MIT
28S_Mat_qPCR_R	CGCCCGACCCCGCGGACGGGGAAG			
28S_Som_qPCR_F	CGGCGCCCCCTCTCGTTCCCGTCTCC	KAPA SYBR 68 °C	110 bp from Som28S	MIT
28S_Som_qPCR_R	CCTCCCCACCCGAGGCGGGGGC			

The 18S_ES3S_FLP primer pair was identically and independently designed by both A.N.S and F.L.

*The 28S_ES31L_FLP_F1 primer contains a 5' M13F tail (19-nucleotides) and the 28S_ES31L_FLP_R1 primer contains a 5' PIG tail (6-nucleotides) adding 25 bp to the final product length. These were included to allow a third primer (5' FAM-labelled M13F) to generate fluorescent PCR products for quantification using formamide denaturing capillary electrophoresis (Varshney et al. 2015). Note the 28S PCR FLP in **Fig. 4B** uses pair 1 (generating 254 bp and 210 bp products) while the 28S PCR FLP in **Supplementary Fig. S2B** uses pair 2 (generating 196 bp and 152 bp products).

Additional files

Movie 1. Map of the zebrafish maternal ribosome isolated from 6 hpf embryos (PDB: 7OYB; Leesch and Lorenzo-Orts et al., 2023). Maternal rRNA sequences are shown in cartoon representation in shades of yellow (see Fig. 3A for the color code); locations of nucleotide differences in zebrafish somatic rRNAs are highlighted in blue (predominantly substitutions), red (predominantly deletions in somatic rRNAs) and purple (substitutions and deletions in somatic rRNAs). [↗](#)

Movie 2. Same as Movie 1, but rRNA sequences are shown in surface representation. [↗](#)

Movie 3. Same as Movie 2, but cross-section through the ribosome. [↗](#)

Supplementary Table 2. Structural comparison of sequence differences between maternal and somatic rRNA variants in zebrafish. rRNA sequence differences between zebrafish maternal and somatic 28S, 18S, 5.8S and 5S rRNAs. Listed are maternal and corresponding somatic rRNA nucleotide positions that differ between the maternal and somatic variants. The table also includes comments based on the analysis of the location and base-pairing status of the specific nucleotides in the 6 hpf zebrafish ribosome structure containing maternal rRNAs. [↗](#)

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Reviewer #1 (Public review):

In all animals, the fertilized egg is transcriptionally silent, and thus early embryonic development relies on maternally deposited factors. A key mode of regulation is translational control to produce the proteins needed by the developing embryo. In zebrafish as well as other animals, distinct ribosomes, those coming from the maternal pool (maternal ribosomes produced in the germ line/oocytes), and those produced from new transcription after genome activation (somatic ribosomes). In zebrafish, the maternal pool consists of a "maternal" rRNA produced from rDNA on chromosome 4, that has previously been shown to be amplified or expressed specifically in the germ line and in oocytes. The observed sex-specific expression of

m-rDNA has led to models that it is involved in sex differentiation and/or maternal control of early embryonic development, both as mediators of translation and as a source of raw materials needed to produce new ribosomes. The work to date in the field indicates that maternal and somatic ribosomes are distinct in their expression profiles but whether they have unique, or gene-specific activities awaits determining if somatic rDNA can functionally replace m-rDNA.

In this manuscript, the authors investigated the expression profiles, protein composition, and ability of maternal and somatic ribosome components to interact with one another and their association with polysomes. This study reports sequence differences between maternal and somatic ribosomal components as well as proteomics and structural analysis of ribosome composition in oocytes and early development. This analysis shows that ribosome subunit composition changes over developmental time but did not uncover evidence suggesting maternal or somatic ribosome-specific ribosomal protein paralog use. The key findings of this work are:

- (1) Observation of hybrid ribosomes composed of subunits of maternal and somatic origin in the embryo.
- (2) Detection of both maternal and somatic ribosomes in polysomes, indicating maternal and somatic ribosomes both support translation in the embryos and may not be functionally unique.
- (3) Persistent expression of m-rRNA in germ cells, suggesting m-ribosomes, as the main ribosome type present, are important for translation in germ cells. The question of ribosome heterogeneity and the function of maternal versus somatic rDNA and ribosomes is of great interest to the broader scientific community. Overall, the manuscript is clearly written and the solid data provided support the main ideas and conclusions.

Specific points are detailed below.

(1) In Figure 1D the m-rRNA abundance goes down at 3dpf, then up again while the s-rRNA steadily increases and peaks at 3dpf then drops thereafter. As presented in the graph it is unclear if this up-then-down trend is consistently observed or not. There are bars on the graph for m-rRNA but not for s-rRNA, thus it is unclear how many times this experiment was performed for the s-rRNA or how variable the results were from sample to sample. Beyond this technical point, if the pattern is consistent, this is an interesting observation as it would signal either a shift in rDNA transcription to silence the somatic locus and/or post-transcriptional targeted degradation of the somatic rRNA in germ cells.

(2) Although qualified by the authors to some extent, the conclusion regarding maternal ribosomes and specificity related to the translation of germ line-specific transcripts is potentially confusing or misleading. Since the maternal form appears to be the only or predominant form of ribosomes in the germ cells at this stage, these would be the only ribosomes available for translation in germ cells. So, any RNA being translated in the germ cells, even RNAs that are not specifically expressed in the germline would be "enriched in association with" and translated by the maternal ribosomes in germ cells. Additional supporting evidence would be required to support the conclusion that the maternal ribosomes are specifically dedicated to the translation of germ cell-specific RNAs, like nanos3, rather than just general translation in germ cells. Consistent with a more general role for the maternal ribosomes in translation in germ cells, differential codon use has been previously documented for the RNAs produced in oocytes (aka maternal RNAs) (for example Bazzini et al EMBO 2016; Mishima and Tomari Mol Cell 2016), and tRNA genes were recently reported by Wilson and Postlethwait to reside along with the maternal 5S genes and maternal-specific spliceosome components in the region of chromosome 4 that is differentially activated in oocytes and testis (region 2 coding genes are silenced in the ovary but maternal ribosome-related genes are expressed in the ovary; region 4 contains the maternal 45S gene). Further, some of the authors of this manuscript undergo a shift in tRNA repertoire and a change in

iso-decoder expression at the onset of gastrulation (Rappol et al, Nucleic Acids Research 2024). Technical limitations pose challenges to definitively testing the hypothesis, but it would be helpful to place the findings here in the context of the published work.

(3) "An alternate and non-exclusive hypothesis is that the maternal rDNA locus may be involved in PGC fate and sex determination in zebrafish." It would be helpful to further discuss the published evidence supporting this hypothesis. In accord with a potential role for m-rDNA in ovary differentiation, differential methylation of m-rDNA has been previously reported, with high methylation in testis and low methylation in ovaries. Further, several groups have shown that treating fish with broad inhibitors of methyltransferases causes testis-biased differentiation of the gonad. Finally, Moser et al (Philosophical Transactions of the Royal Society B 2024) recently published work in which CRISPR-Cas9 was used to target the 45S m-rDNA promoter and interfere with its expression. The mutants with these promoter mutations developed as fertile males, consistent with a role for m-rDNA in ovary differentiation. A recent paper from Moser et. al. (Philosophical Transactions of the Royal Society B 2024) showing that disrupting the m-rDNA locus leads to male-only development should be discussed. This paper does not exclude the possibility of a maternal role for the ribosomes since only one female was recovered among the 45S-m-rDNA mutants. The expression data in Figure 1D of this manuscript showing that m-rRNA levels go down and then up in PGCs indicates the PGCs are making their own m-rRNA. This observation together with the recovery of fertile males reported in the Moser et al study (Philosophical Transactions of the Royal Society B 2024) doesn't seem to support a requirement for m-rDNA in PGC fate or germ cell-specific translation, at least in testis, since the mutant males produce sperm and are fertile.

(4) Although the rationale for examining rRNAs in adult tumors, cultured zebrafish cell lines, and during fin regeneration is clear based on the published literature showing elevated embryonic rRNAs, this line of investigation doesn't add much to this study and is a bit of a distraction. That said, the observation that in contrast to published work, neither the maternal (early embryo) nor the specific rRNAs examined are unregulated in these contexts is important and warrants communication with the research community.

(5) The numbers of embryos and stages are not consistently stated in the manuscript. For example, in the "Isolation of zebrafish ribosome." and "isolation of monosomes" sections of the methods, the stage and number of embryos used for the IPs are not clearly stated in the methods. These important details should be stated throughout the manuscript so that others can perform future studies in a manner that will facilitate comparisons.

(6) The terminology used for the RiboFLAG experiments is potentially confusing or misleading. Specifically, different terms are used to describe the source of the ribosomes (Figure 5, Figure S7, Figure S8 and in the text). For example, "transmission" is used to describe "maternal transmission" for Mat-RiboFLAG, and "paternal transmission" is used for Som-RiboFLAG, and in Figure 5 and Figure S8 "maternally provided" and "paternally provided" are used. However, these terms may be confusing or unintentionally misleading because transmission and provided refer to two different things. In the case of Mat-RiboFLAG, the terms refer to the maternal Rpl10-FLAG ribosomes, which the progeny receive from their mother independent of whether or not they express the transgene. On the other hand, for Som-RiboFLAG, the terms refer to the transgene rather than the Rpl10-FLAG ribosomes that will be produced by the embryo using the transgene they inherited from their father. Consider instead sticking to "maternal" and "somatic", or alternatively "zygotic expression" and "maternal expression" or "zygotic ribosomes" and "maternal ribosomes".

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Reviewer #2 (Public review):**Summary:**

The study expands previous knowledge on the dual ribosome system in zebrafish by demonstrating the expression of maternal ribosomes in the primordial germ cells as well as the formation of hybrid ribosomes combining subunits of maternal and somatic ribosomes. Although the distinction between the two types is clear at the rRNA level, this is not paralleled at the protein level. An attempt to associate the expression of germ-line-specific transcripts to maternal ribosomes remains inconclusive. Thus, evidence for the functional specialisation of ribosomes in this system is still lacking.

Strengths:

The experiments are well-conducted and the main conclusions are well-supported.

Weaknesses:

The attempt to take advantage of the system to provide an example of functional ribosome specialisation is justified and the expression of maternal-type ribosomes in the germ line may still be key to understanding the expression of classes of mRNA. However, an alternative possibility related to genome evolution and sex determination is equally relevant.

Assessment following the structure of the manuscript:

Shah et al.: "A dual ribosomal system in zebrafish soma and germline"

The zebrafish dual ribosome system is attractive because it offers a favourable setting to look for ribosome specialization and my impression is that this is exactly what the authors set out to do rather than to try to understand why zebrafish have this unusual setup. If this is correct, the title and the abstract should better reflect the authors' aim and main results. The title suggests to the non-specialist that the dual ribosome system is a novel find which obviously is not the case.

I was a bit confused when reading the introduction. In the first paragraph, it was unclear to me if the degradation of maternal ribosomes is an active process different from normal turnover. I also found the third paragraph slightly out of tune with the discussion section. The dual ribosome setting at the level of ribosomal RNA genes represents an extreme case of sequence heterogeneity and appears to be sporadic in nature in that it only is reported from *Plasmodium* and zebrafish. The *Xenopus* example is 5S rRNA (as also mentioned in the discussion section), and the *Drosophila* example is protein composition, only. If a broader view of ribosome types is intended, there will be more examples, e.g. Trypanosomes that express different stage-dependent ribosomes at the level of rRNA modifications. The occurrence of dual ribosomes in fish should be placed in context with insight from other fish genomes, e.g. Medaka, which has only one type of ribosomes. Also, the duality in zebrafish is not restricted to ribosomes, but also comprises two types of spliceosomes. These observations suggest that the phenomenon should be investigated in the context of genome evolution. This is appropriately brought up in the discussion section, but I believe it would serve the reading of the manuscript if this was made clear from the beginning. With respect to the structural aspects, I am puzzled why one of the few other papers studying this system, Ramachandran et al. RNA 2020 (PMID: 32912962) is not referenced. This paper is focused on ribose methylation of the two types of ribosomal RNA and should be relevant to several aspects of the present study.

The manuscript reports three novel and important findings. First, the maternal-type ribosomes are expressed in PGCs, where they furthermore are shown to translate germ line-specific transcripts, and in the male germ line. Regardless, the authors wisely decide to

maintain the classical terminology of maternal and somatic ribosomes. Second, both types of ribosomes are polysome-associated and thus translationally active at 24 hpf when they are found in equal amounts. An elaborate experiment shows that hybrid ribosomes are formed at this stage. Finally, a RIP experiment fails to show selectivity in ribosomal recruitment of a germ line-specific mRNA based on the nanos3 3'-UTR. There are several other results, but these are mainly confirmatory or negative, albeit of good quality and important to communicate.

The part of the study that describes differences in protein composition is a bit difficult to follow, partly because of the complexity of the results, and partly because of the disappointment that no parallel changes in proteins to the clear differences in rRNA were observed. Except for the discussion of eS8 in relation to subunit bridging, it is purely descriptive. There is quite a literature on paralog expression (e.g. in yeast and humans) and perhaps it would be possible to relate to the literature in a way that could provide more meaning to the observations. From the M&M section, it appears that the proteomics data were already published in the Leesch and Lorenzo-Orts et al. paper (Nature 2023). They are here found in Table S1 which is presented in a minimal fashion, from which it is time-consuming to extract meaningful information, e.g. on how stringently the ribosomes were prepared.

The hybrid-ribosome observation is convincing, but additional information on the choice of cycloheximide concentration would be helpful to rule out other interpretations.

The experiment on translation of primordial germ cell-specific transcripts by maternal ribosomes is a key experiment. Unfortunately, the experiment failed to show selectivity compared to somatic ribosomes, and in my reading, the promise in the abstract of "preferential association" is not quite justified. More importantly, this experiment is not exhaustive, and a more elaborate discussion on the limitations of the experiment and other approaches would be helpful.

The discussion section is interesting. Importantly, the authors make the non-specialist aware of the peculiarities of laboratory strains of zebrafish with respect to the lack of sex chromosomes and a possible connection between the rDNA locus and sex determination. This information is critical to include in a journal that has a broad readership. I was unable to follow the argument about the 3' half of 5.8S "to play a role" in ribosome degradation based on Locati et al., 2018 (which is missing from the reference list) and "serve as a target for degradation of maternal ribosomes". Kinetic effects on the degradation pattern of rRNA are frequently observed and difficult to interpret.

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Reviewer #3 (Public review):

Summary:

Ribosomes are generally considered homogeneous complexes with no inherent role in regulating translation. However, recent studies have found heterogeneity in the composition of ribosome accessory factors, proteins, and ribosomal RNA. Moreover, there is evidence that distinct ribosomal isoforms are produced at different developmental stages in *Xenopus*, *Drosophila*, and zebrafish. In *Drosophila*, germline-derived ribosomes have a different protein composition to those produced by somatic cell types. In zebrafish, germline vs. somatic ribosomes have been shown to incorporate distinct rRNA isoforms. However, the functional significance of ribosome heterogeneity is not known.

The manuscript by Shah et al., uses the power of the zebrafish to test the hypothesis that maternal ribosome isoforms have a distinct function relative to ribosome isoforms produced by somatic cells after the maternal-to-zygotic transition (MTZ). They confirm previous findings that all maternal rRNA are derived from the maternal-specific rRNA locus on Chromosome 4. Additionally, proteomic analysis showed that maternal and somatic ribosomes also differ in protein composition. Using ribosome tagging experiments they showed that maternally derived subunits can form functional heteroduplexes (hybrids) with somatic-derived subunits. Finally, they show that maternal-derived ribosomes continue to be expressed in germ cells where they preferentially associate with the maternally derived and germline localized nanos3 mRNA. This suggests a possible role of maternal ribosomes in germ cell-specific translational regulation.

Strengths:

The authors use the experimental power of zebrafish to test the hypothesis that maternal and somatic-derived ribosomes have distinct functions. They use state-of-the art proteomics, molecular modeling, and transgenesis techniques. For the most part, the data presented is clear and supports their conclusions.

Weaknesses:

Using pulldown experiments they show that maternal ribosomes associate with the PGC-enriched nanos3 RNA, suggesting a role for the maternal isoform in germline-specific translation. However, they acknowledge that the level of enrichment is similar to the level of maternal vs. somatic isoforms that localize to PGCs. The nanos3 mRNA is unique in that it is actively degraded in somatic cells shortly after MTZ so is never present in cells that express the somatic isoforms. Therefore, the association of nanos3 with maternal ribosomes shows that these ribosomes can associate with germline-specific RNAs, but does not provide compelling evidence for a maternal isoform-specific role in translational regulation.

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