A molecular network of conserved factors keeps ribosomes dormant in the egg

Ribosomes are produced in large quantities during oogenesis and are stored in the egg. However, the egg and early embryo are translationally repressed1-4. Here, using mass spectrometry and cryo-electron microscopy analyses of ribosomes isolated from zebrafish (Danio rerio) and Xenopus laevis eggs and embryos, we provide molecular evidence that ribosomes transition from a dormant state to an active state during the first hours of embryogenesis. Dormant ribosomes are associated with four conserved factors that form two modules, consisting of Habp4-eEF2 and death associated protein 1b (Dap1b) or Dap in complex with elf5a. Both modules occupy functionally important sites and act together to stabilize ribosomes and repress translation. Dap1b (also known as DapII in mammals) is a newly discovered translational inhibitor that stably inserts into the polypeptide exit tunnel. Addition of recombinant zebrafish Dap1b protein is sufficient to block translation and reconstitute the dormant egg ribosome state in a mammalian translation extract in vitro. Thus, a developmentally programmed, conserved ribosome state has a key role in ribosome storage and translational repression in the egg.

Ribosomes are among the most abundant macromolecular complexes stored in the quiescent egg5. These maternally provided ribosomes (hereafter referred to as maternal ribosomes) are essential for the translation of maternal and zygotic transcripts5-9 (Fig. 1a). Although the overall number of ribosomes per embryo remains constant throughout early embryogenesis, previous studies in multiple organisms indicate that translational activity increases in the embryo, suggesting that translation repression in the egg is temporarily dampened10-13. Several mechanisms, including shortening of mRNA polyadenine tails12 and interference with the formation of the translational initiation factor elf4F13, have been implicated in dampening translation in the egg and early embryo; however, to our knowledge, there is no direct evidence for a contribution of the ribosome itself.

Translationaly inactive ribosomes have been observed in prokaryotes and eukaryotes in specific cellular contexts14. Under stress conditions, prokaryotic 70S ribosomes dimerize to form 100S particles via the association of hibernation promoting factor (HPF) and ribosome modulation factor (RMF) in Gram-negative bacteria15, or long form hibernation promoting factor (LHPF) in Gram-positive bacteria16. In eukaryotes, several factors have been reported to associate with inactive ribosomes upon sporulation (such as MDF1 and MDF2 in microsporidia17) and nutrient deprivation. The latter include SERBP118-21 which are known to inhibit translation by inserting into the polypeptide exit tunnel of bacterial ribosomes.

Studies from the 1970s with sea urchin eggs indicated the presence of inhibitory proteins that were thought to be associated with maternal ribosomes22-24, yet the relevance and molecular identity of these factors remained unclear. How ribosomes are stored in an inactive state for extended amounts of time in the mature egg, and whether regulation of the ribosome itself contributes to translational repression in the egg and the subsequent increase in translational activity during embryogenesis, thus remain unknown.

Translation is repressed in zebrafish eggs

To investigate the timing of translational activation during the egg-to-embryo transition in vertebrates, we examined the translational status of zebrafish embryos at different stages of development (Fig. 1a). Specifically, we used polysome gradients to uncover the fraction of ribosomes present as individual subunits (40S and 60S), monosomes (80S) and polysomes. In the absence of triggers of ribosome stalling, an increase in the polysome fraction generally indicates increased active translation25. We found that ribosomes in the egg and in the one hour post-fertilization (hf) embryo were almost exclusively present as monosomes, whereas the polysome fraction started to increase from 3–6 hpf onwards (Fig. 1b,c). As an orthogonal approach, we calculated translational efficiency values based on ribosome-protected mRNA fragments over the course of zebrafish embryogenesis, using published ribosome profiling and RNA-seq datasets30,31. We observed that...
Identification of dormant ribosome factors

To uncover factors that regulate maternal ribosome activity during the first hours after fertilization, we purified crude ribosomes (monosomes and polysomes) from zebrafish eggs and embryos for mass spectrometry analysis. Although the relative abundances of core ribosomal proteins were similar at all time points, we identified four evolutionarily conserved factors, namely the paralogues eIF5a/eIF5a2, eEF2b, Habp4 and the paralogues Dap1b/Dap that were specifically associated with ribosomes in zebrafish eggs and 1 hpf embryos, but depleted from ribosomes in 3 hpf and 6 hpf embryos (Fig. 1e,f and Supplementary Table 1). The levels of these ribosome-associated factors remained relatively stable in total embryo lysates over the first 6 h of development (Fig. 1f), suggesting that they were released from the ribosome...
but not immediately degraded. Of note, we found that the same set of factors were enriched in ribosomes purified from *Xenopus* eggs and 24 hpf embryos (a developmental stage equivalent to zebrafish 6 hpf embryos) (Extended Data Fig. 1a). Thus, the association of these four factors with ribosomes in the mature egg is conserved in zebrafish and *Xenopus* and correlates with suppressed translation.

Structure of the dormant egg ribosome

To explain how these factors establish a translationally repressed ribosome state, we determined the structures of maternal ribosomes isolated from 1 hpf zebrafish embryos (Fig. 2a) and *Xenopus* eggs (Fig. 2b) by cryo-electron microscopy (cryo-EM) at 3.2 and 2.8 Å average resolution.
respectively (Extended Data Figs. 1b–f and 2 and Supplementary Table 2). We were able to assign densities for all four factors in ribosomes from both zebralfish and *Xenopus* (Fig. 2c and Extended Data Fig. 3a–d). In parallel, we performed cryo-EM analysis of ribosomes isolated from 6 hpf zebralfish embryos (2.6 Å average resolution; Extended Data Figs. 3e and 4 and Supplementary Table 2). In agreement with the mass spectrometry data, ribosomes from 6 hpf zebralfish embryos showed low density levels for eEF2 and no densities for eIF5a or eIF5a2, Habp4 and Dap1b or Dap (Extended Data Figs. 3e and 4).

eIF5a and eEF2 are known essential translation factors. eIF5a normally promotes translation elongation and termination, particularly upon ribosome stalling at specific amino acid sequence contexts. We found that eIF5a or eIF5a2 binds between the exit (E) and peptidyl (P) site of the ribosome (Fig. 2a,b), as previously reported for a stalled ribosome. eEF2 mediates ribosomal translocation and transiently interacts with the tRNA–mRNA complex at the aminoacyl (A) site of the ribosome. The eEF2 interaction at the A site has been reported to be stabilized in inactive ribosomes by proteins of the H4pap family, including Suppressor of tom1 (Stm1) in yeast and SERPINE1 in mammals; but not by Habp4 itself. Indeed, we found Habp4 bound to the mRNA-entry channel of zebralfish 1 hpf and *Xenopus* egg ribosomes that we could attribute to the conserved C-terminus of Dap1b/Dap1 or Dap (Fig. 2e and Extended Data Fig. 3c,d). Dap1b was about tenfold more abundant than Dap in ribosomes from 1 hpf zebralfish embryos according to our mass spectrometry analysis (Fig. 1f), and only Dap1 was identified by mass spectrometry in ribosomes from *Xenopus* eggs (Extended Data Fig. 1a). Thus, we modelled the C-terminus of zebrafish Dap1b and *Xenopus* Dap, which we found to extend by five amino acids beyond the position that is occupied by the C-terminal amino acid residue of a nascent polypeptide chain (Fig. 2c,f). The fifth-last invariant amino acid in Dap1b/Dap1 (Q105 in zebrafish and Q109 in *Xenopus*; Extended Data Fig. 5a) occupies the same position as the C-terminal residue of a nascent polypeptide chain and forms a hydrogen bond with the hypusine residue of eIF5a (Fig. 2f), which is essential for eIF5a function. Moreover, we observe additional hydrogen-bond interactions between the immediately adjacent glutamine in Dap1b/Dap1 (Q104 in zebrafish and Q108 in *Xenopus*) with the 28S rRNA (Fig. 2f,g). Notably, the interaction of this glutamine with A3073 in *Xenopus* (A3168 in zebrafish) restricts this adenosine to a conformation distinct from those in previously reported ribosomal structures (Fig. 2g). Moreover, the path of Dap1b/Dap1 differs from those of other proteins that have previously been found to be inserted into the PET of mature or precursor large ribosomal subunits, including antimicrobial peptides (such as Bαc21 and AplI324–26), biogenesis factors (such as RelI and NogS27–29) and the dormancy factor MDF220 (Extended Data Fig. 6). Our structural analyses suggest that the specific interactions of Dap1b/Dap1 with eIF5a and the 28S rRNA contribute to the binding of Dap1b/Dap1 to the PET of a fully assembled eukaryotic 80S ribosome, thus making them unique among the characterized ribosome-associated factors.

All four factors bind to the same ribosome

To determine whether the four factors are bound to the same ribosomal particles, we performed a 3D variability analysis using cryoDRGNN48, each dataset’s latent representation showed distinct clusters when visualized by uniform manifold approximation and projection (UMAP) (Extended Data Fig. 3f). A detailed analysis of the particles in these clusters revealed six major classes based on the presence or absence of eEF2, eIF5a and tRNAs (Supplementary Table 3). In contrast to actively translating ribosomes, which show mutually exclusive binding of eIF5a and eEF223,24,29, our analysis revealed that 40.2% and 26.9% of ribosomes in *Xenopus* eggs and in 1 hpf zebralfish embryos, respectively, were simultaneously bound by eIF5a and eEF2 (Fig. 2h, class I particles). The reconstruction of a high-resolution map from *Xenopus* egg class I ribosomes revealed that Dap1b and Habp4 are also present (Fig. 2h and Extended Data Fig. 3g,h). Given that class I ribosomal particles were exclusively identified in eggs and early embryos, which are characterized by a low translational activity (Fig. 1b–d), we will refer to these ribosomes as ‘dormant.’ Other ribosomal particle classes, including class II particles containing only eEF2, class III particles containing tRNAs, and ‘empty’ class IV particles lacking eEF2, eIF5a and tRNAs were identified to varying levels in all datasets (Fig. 2h and Supplementary Table 3). We hypothesize that ‘empty’ class IV particles may correspond to ribosomes that have lost ribosome-associated factors or tRNAs during the purification as they do not cluster together in the UMAPs (Extended Data Fig. 3f). Together, our results reveal a novel ribosome state in which four factors (Habp4, eEF2, Dap1b/Dap1 and eIF5a) bind to the same ribosome.

Crosslinking of dormant ribosomes

Although we could assign cryo-EM densities for small amino acid stretches within Habp4 and Dap1b/Dap1 and Dap proteins, the majority of the polypeptide chains of these factors were not visible in our ribosome structures. To determine the path of these novel ribosome-associated proteins within the ribosome, we performed crosslinking–mass spectrometry with the primary amine crosslinker disuccinimidyl sulfoxide (DSSO) using ribosomes purified from 1 hpf zebralfish embryos and *Xenopus* eggs (Extended Data Fig. 7 and Supplementary Table 4). We obtained more than 1,000 crosslinked peptides for each ribosome sample, and 95% (zebrafish) and 90% (*Xenopus*) of all mapped crosslinks had Cα–Cα distances below the expected maximum crosslinking length28 of 23 Å (Extended Data Fig. 7a). We found crosslinks of the N terminus of Habp4—which was not visible in the cryo-EM densities—with small and large subunit ribosomal proteins, including Rpl7a (also known as eL8) and Rps3a (also known as eS1), in dormant ribosomes from both zebralfish and *Xenopus* (Extended Data Figs. 7b,d and Supplementary Table 4). Crosslinking–mass spectrometry analyses of Dap1b/Dap1 and Dap showed that their N-terminal regions are proximal to the polypeptide exit site, which is consistent with insertion of their C termini into the PET. In particular, we found crosslinks between a highly conserved N-terminal region of Dap1/Dap1 and Rpl31 (also known as eL31), and between the central region of Dap1/Dap and Rpl35 (also known as eL29) (Extended Data Figs. 7c,e and Supplementary Table 4), indicating that both N- and C-terminal regions of Dap1/Dap1 and Dap are in close proximity to the ribosome.

In vivo role of dormant ribosomes

Zebrafish and *Xenopus* habp4, dap1b/dap1l, dap and eif5a2 mRNAs are highly expressed during oogenesis, whereas eif5a and eef2 transcripts are abundant in all tissues (Extended Data Fig. 5b–d). Moreover, Habp4 family proteins have been linked to ribosome stabilization during nutrient deprivation in yeast23 (Stm1) and to non-translating ribosomes in yeast24,25, *Drosophila*19 (Vig2) and mammals,18,21,22,38 (SERBP1). On the basis of these observations, we hypothesized that Habp4 and Dap1b/Dap1/Dap have key roles in the prolonged ribosomal storage and/or translational repression in the egg. To test this hypothesis, we used CRISPR–Cas9-based mutagenesis to generate individual (hapb4−/−),
double (dap\(^{-/^{-}}\) dap1b\(^{-/^{-}}\)) and triple mutant (dap\(^{-/^{-}}\) dap1b\(^{-/^{-}}\) habp4\(^{-/^{-}}\)) zebrafish lines (Supplementary Fig. 1a–c).

Habp4\(^{-/^{-}}\) mutants were viable and showed normal egg clutch sizes, fertility, embryo development and survival rates (Extended Data Fig. 8a,c,d,f–h). However, 1–3 hpf embryos derived from habp4\(^{-/^{-}}\) parents contained 30% fewer monosomes as well as about 30% less total RNA (80–90% of the total cellular RNA is estimated to be ribosomal RNA) than their wild-type siblings (Fig. 3a–c). This molecular phenotype could be rescued by oocyte-specific transgenic expression of Habp4 (Fig. 3a and Supplementary Fig. 1d). In contrast to the reduction in ribosomes observed in habp4\(^{-/^{-}}\) embryos, no significant difference was observed between polysome levels in 1 hpf habp4\(^{-/^{-}}\) and wild-type embryos (Fig. 3c and Supplementary Fig. 1k). These findings demonstrate that Habp4 functions in stabilizing ribosomes. Moreover, the lack of an overt developmental phenotype in habp4\(^{-/^{-}}\) embryos suggests that ribosomes are stored in excess in the zebrafish egg given that a
reduction in ribosome levels had previously been shown to cause development defects (for example, minute phenotypes in Drosophila) and to alter cell differentiation (for example, in embryonic stem cells and tumour formation in adult zebrafish).

Adult dap⁻/⁻ dap1b⁻/⁻ fish were obtained from a double heterozygous parent in-cross (dap⁺⁻/⁻ dap1b⁺⁻) at sub-Mendelian ratios compared with wild-type siblings (Fig. 3d), indicating a fitness defect. Although surviving dap⁻/⁻ dap1b⁻/⁻ adults appeared morphologically normal and showed normal egg clutch sizes, fertility, and embryo development and survival rates (Extended Data Fig. 5b–f), their progeny displayed a significantly higher polysome-to-monomosome ratio at 1 hpf compared with 1 hpf wild-type embryos (Fig. 3e,f). This ratio was similar to that observed for 2 hpf and 3 hpf wild-type embryos (Fig. 3f). Transgenic overexpression of Dap1b in dap⁻/⁻ dap1b⁻/⁻ embryos was sufficient to rescue the higher polysome-to-monomosome ratio observed in these mutants (Fig. 3f and Supplementary Fig. 1e–i), pointing to a role for Dap1b in translational inhibition. Of note, dap⁻/⁻ dap1b⁻/⁻ embryos did not show differences in monosome levels (Supplementary Fig. 1j), which suggests that in contrast to Habp4, Dap1b and Dap are not important for ribosome stabilization in the egg.

Given that Habp4 and Dap/Dap1b associate with the same ribosomes and contribute to their dormancy, we generated triple-knockout (KO) (dap⁻/⁻ dap1b⁻/⁻ habp4⁻/⁻) mutants to assess the physiological importance of the dormant egg ribosome state. Whereas eggs and embryos derived from habp4⁻/⁻ and dap⁻/⁻ dap1b⁻/⁻ mutants developed without overt morphological abnormalities, the absence of all three proteins in adult triple-KO females resulted in impaired oogenesis and embryogenesis (Fig. 3g). On average 30% of the eggs laid by triple-KO females were of poor quality (Extended Data Fig. 5g) and of the remaining 70%, only 74% (93% for wild-type eggs) were fertilized and showed proper early embryo development (Extended Data Fig. 5h). Moreover, an increased fraction of embryos from triple-KO fish showed severe abnormalities by 1 and 4 days post-fertilization, including abnormal head and eye development (Extended Data Fig. 5i,j). Taking into consideration all oogenesis and embryogenesis defects, less than 50% of eggs from triple-KO fish developed into phenotypically normal larvae (Fig. 3g). Notably, these defects are owing to the lack of dormancy factors in the egg (maternal phenotype) as these phenotypes were only observed in embryos derived from crosses with triple-KO females (Extended Data Fig. 5h–j). Analysis of rRNA and monosome content in developing triple-KO embryos revealed a reduction in the amount of ribosomes compared to the wild type (Fig. 3h and Extended Data Fig. 5e). Notably, whereas dap⁻/⁻ dap1b⁻/⁻ embryos also show a trend towards more active translation per embryo (normalized to α-tubulin), translation levels per embryo in habp4⁻/⁻ and triple-KO samples resemble the wild type (Fig. 3i). Together, our in vivo data suggest that Habp4 and Dap/Dap1b may have distinct roles in ribosome dormancy, with Habp4 contributing to ribosome stability and Dap/Dap1b contributing to translational repression. However, a role for Habp4 in translational repression cannot be ruled out at this stage.

**Dap1b represses translation in vitro**

To directly assess the functions of Dap1b/Dap1l and Dap in translational repression independently of other layers of translational regulation present in vivo, we performed in vitro translation assays in rabbit reticulocyte lysate (RRL) in the presence of recombinant Dap and Dap1b proteins (Fig. 4a). Dap and Dap1b/Dap1l are small (approximately 15 kDa), unstructured proteins that are rich in basic amino acids and prolines (15%). Invertebrates contain only one orthologue, whereas vertebrates have evolved two paralogues (Extended Data Fig. 5a). Despite displaying less than 50% overall sequence identity, all Dap1b/Dap1l and Dap proteins share two highly conserved motifs at the N and C termini, which we found proximal to Rpl31 (Extended Data Fig. 5a and 7c,e) and within the PET (Fig. 2e,f), respectively. Whereas recombinant zebrafish Dap or the negative control BSA did not affect the translational activity of RRL, recombinant zebrafish Dap1b repressed the translation of a luciferase mRNA to a similar extent as the antimicrobial peptide Bac7 (Fig. 4b), which is known to bind to the PET and inhibit translation23. To investigate whether the observed difference in repressive activity between zebrafish Dap and Dap1b was generalizable to other Dap and Dap1b/Dap1l proteins, we assessed ribosomal binding of in vitro translated tagged dap and dap1b/dap1l mRNAs from various species (Fig. 4a). Consistent with a general difference in repressive activity between Dap and Dap1b in vitro, Dap showed a weaker affinity for rabbit ribosomes than Dap1b/Dap1l from three different vertebrate species (Fig. 4c,d and Extended Data Fig. 9a). Analysis of ancestral Dap1 proteins from invertebrates revealed that Dap from Caenorhabditis elegans and from the coral Pocillopora had an affinity for rabbit ribosomes in between that observed for vertebrate Dap and Dap1b proteins, whereas Dap from Drosophila melanogaster did not bind to rabbit ribosomes (Fig. 4c,d and Extended Data Fig. 9a).

We noted that all rabbit ribosome-binding vertebrate Dap1b/Dap1l proteins as well as the ancestral Pocillopora and C. elegans Dap1 proteins contain at least one additional residue at the C terminus compared with non-ribosome-binding vertebrate and Drosophila Dap1 proteins (Extended Data Fig. 5a). However, extending zebrafish Dap or shortening zebrafish Dap1b by one amino acid did not alter their ribosome-binding capability (Fig. 4e,f andExtended Data Fig. 9b). Instead, mutating the conserved, hypusine-interacting Q105 of zebrafish Dap1b (as well as the corresponding Q102 of zebrafish Dap) abolished the binding of Dap1b (and Dap) to the ribosome (Fig. 4e,f and Extended Data Fig. 9b). Thus, whereas the invariant Q105 (Xenopus Q109) is key in stabilizing Dap1b binding to the ribosome, the Dap1b C terminus (residues 76–109) alone was not sufficient to repress translation (Extended Data Fig. 9c). This is in contrast to the similarly short peptide Bac7, suggesting that both N- and C-terminal regions of Dap1b are important for its insertion into the PET. In agreement with this conclusion, chimeric proteins formed by the N terminus of Dap and the C terminus of Dap1b (and vice versa) bound less efficiently to the ribosome than Dap1b, but more efficiently than Dap (Extended Data Fig. 9d). Together, these analyses revealed that Dap1b/Dap1l but not Dap binds efficiently to mammalian ribosomes in vitro and is sufficient to inhibit translation.

**Reconstitution of ribosome dormancy**

To assess whether Dap1b was inserted into the PET of mammalian ribosomes, and whether Dap1b addition to translational extracts indeed reconstitutes the dormant ribosome state, we performed cryo-EM on ribosomes isolated from an in vitro translation extract supplemented with 20 μM of recombinant zebrafish Dap1b, obtaining a map with a 2.3 Å average resolution (Fig. 4g, Extended Data Fig. 10a,b and Supplementary Table 2). Notably, we were able to assign densities not only for Dap1b in the PET, but also for the other dormant ribosome factors—namely elf5A, elf5E and the H4b-homologue SERBP1 (Fig. 4g and Extended Data Fig. 10c)—that are present in RRL24. A 3D classification of these ribosomal particles using cryo-DRGN revealed that, after addition of zebrafish Dap1b, 56% of particles were in a dormant state similar to ribosomes from 1 hpf zebrafish embryos and Xenopus eggs (Fig. 4h, class 1). Extended Data Fig. 10d and Supplementary Table 3. Although several cryo-EM structures of ribosomes isolated from RRL have been published, none of them has reported the presence of elf5A.
and eEF2\textsuperscript{18,36}, which is consistent with Dap1b being causally linked to this dormant ribosome state. Although Dap1b is required to generate the dormant ribosome state, the absence of Dap1b in zebrafish embryos resulted only in a decreased ribosome association of eIF5A but not of eEF2 and Habp4 (Extended Data Fig. 9e). By contrast, ribosomes isolated from h\textsuperscript{47} embryos showed decreased levels of all three proteins (Extended Data Fig. 9e), suggesting that Habp4 is required for binding of the other dormancy factors. Although this reduction did not reach significance, the extent of the effect could be masked by two Habp4 paralogues, Serbp1a and Serbp1b, which are associated with ribosomes from h\textsuperscript{47} embryos (Extended Data Fig. 9e) and might partially compensate for the loss of Habp4 in these embryos.

Discussion

Together, our results support a novel, dormant ribosome state present in the mature egg in which four functionally important sites of the ribosome—the A site and the E and P site, the mRNA channel and the PET—are occupied by conserved factors (Fig. 4). Based on direct protein interactions, these factors are organized in two modules: the Habp4–eEF2 module and the Dap1b/Dap–eIF5a module. In vivo and...
in vitro data revealed Daplb as a translational repressor. Although Hapb4 binding to the mRNA-entry channel is also incompatible with translation, our in vivo data point to a main function for Hapb4 in stabilizing ribosomes rather than in repressing translation. Notably, two Hapb4 paralogues—Serbp1a and Serbp1b—are associated with ribosomes at 1 hpf and might contribute to translational repression in hapb4 mutants. Overall, both modules are important for establishing ribosome dormancy as loss of Dap, Dapb and Hapb4 in triple-KO embryos results in a reduced amount of dormant ribosomes and strong developmental phenotypes. Establishing ribosome dormancy during oogenesis might thus be important for the increase in translation upon fertilization, which we speculate to be achieved via the release of dormant factors from the ribosome. Alternatively, dormant ribosomes might have additional roles in protecting the essential translational factors elf5a and elfe2, which have been shown to be prone to aggregate with aging46, and/or serve as a source of pyrimidines to maintain nucleotide homeostasis in the embryo (as proposed for C. elegans).

Although ribosome storage is notably different in the egg compared to any other so far described inactive ribosome state in somatic cells, similar mechanisms could potentially be used at other stages in the lifetime of animals that require prolonged periods of low metabolic activity (such as the dauer stage in C. elegans). The mechanism that we describe here, with functionally important sites of the ribosome occupied by evolutionarily conserved factors, two of which are needed for subsequent resumption of translation (Fig. 4), appears ideally suited to accomplish energy-preserving long-term storage of ribosomes in a variety of developmentally programmed and metabolically induced contexts.

Online content
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Xenopus globin

**Methods**

**Zebrafish and Xenopus lines and husbandry**

Zebrafish (D. rerio) were raised according to standard protocols (28 °C; 14:10 h light:dark cycle). TLAB fish, generated by crossing zebrafish AB with the natural variant TL (Tupfel Longfin), served as wild-type zebrafish for all experiments. Zebrafish dap, dap1b and hapb4 mutants were generated as part of this study and are described below. Wild-type X. laevis were obtained from NASCO and maintained in the IMP animal facility. All fish and Xenopus experiments 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 59-221180-2021-16; animal protocols for work with Xenopus: BMWFW-66.006/0012-WF/II/3b/2014, BMWFW-66.006/0003-WF/II/3b/2016). Zebrafish were grouped based on their genotype and age. In vivo samples were allocated randomly to the experiment and treated equally.

**Generation of knockout and transgenic fish lines**

Zebrafish dap, dap1b and hapb4 mutants were generated by Cas9-mediated mutagenesis. A pool of two single guide RNAs (sgRNAs) targeting the first exon of either dap (chr24:22071216-22103555), dap1b (chr9:52378482-52386733) or hapb4 (chr21:20396891-20402554) (chromosome locations are based on GRCz11) were generated according to published protocols (see Supplementary Table 5 for oligonucleotides used for generating sgRNAs). Cas9 protein and sgRNA pools were co-injected into one-cell stage TLAB embryos. Putative founders were outcrossed to TLAB, and germline mutations were identified by a size difference in PCR amplicons (see Supplementary Table 5 for primers used for genotyping). Embryos from founder fish were raised to adulthood. Homozygous knockout fish were generated by crossing heterozygous fish. To generate dap−/− dap1b−/− dapb4−/− double mutants, dap−/− and dap1b−/− fish were crossed, and double heterozygous mutants were identified by genotyping and raised to adulthood. Double homozygous mutants (dap−/− dap1b−/−) and wild-type (dap+/+ dap1b+/+) siblings were obtained from in-crossing double heterozygous mutants. To generate dap−/− dap1b−/− hapb4−/− triple mutants, dap−/− dap1b−/− and hapb4−/− fish were crossed, and triple homozygous mutants were identified by genotyping and raised to adulthood. Triple homozygous mutants (dap−/− dap1b−/− hapb4−/−) and double mutants (dap−/− dap1b−/− hapb4−/− and dap−/− dap1b−/− hapb4−/−) and wild-type siblings were obtained from in-crossing double heterozygous mutants.

To generate N-terminally tagged 3×Flag-sfGFP-Dap or 3×Flag-sfGFP-Dap1b transgenic fish lines, the respective open reading frame (ORF) was amplified by PCR from zebrafish cDNA and cloned via the Gibson assembly method and BamHI and EcoRV sites into a vector for Tol2-based transgenesis containing the actb2 promoter and 5′ untranslated region (UTR), an SV40 3′ UTR and an ampicillin resistance gene (final constructs: Tol2-actb2-3×Flag-sfGFP-Dap - SV40 3′ UTR; Tol2-actb2-3×Flag-sfGFP-Dap1b - SV40 3′ UTR).

Plasmids (15 pg) were injected together with 35 pg Tol2 mRNA into the cell of 1-cell stage TLAB embryos. Putative founders were crossed to the dap−/− dap1b−/− line, and transgenic offspring were identified by GFP expression and genotyped for dap and dap1b mutations at 8 weeks. Adult fish expressing the transgene and heterozygous for dap and dap1b were incrossed. Larvae from this cross were screened at 2 days post-fertilization for GFP expression and genotyped for dap and dap1b mutations at 8 weeks.

To generate a C-terminally tagged Hapb4-3×Flag transgenic fish line, the respective ORF lacking the STOP codon was amplified by PCR from zebrafish cDNA (RNA from multiple embryonic stages was pooled) and cloned via the Gibson assembly method and XmaI and NheI sites into a vector for Tol2-based transgenesis containing the zp3 promoter, a Xenopus globin 5′ UTR, an SV40 3′ UTR, a cmilc2:GFP as transgenesis marker and an ampicillin resistance gene (final constructs: Tol2 - zp3: globin 5′ UTR - habp4-3×Flag - SV40 3′ UTR, cmilc2: GFP). The plasmid was injected into embryos as described above. Putative founders were crossed to habp4−/− fish and transgenic offspring were identified by screening for cmilc2-driven heart-specific expression of GFP at 30 hpf.

**Phenotypic characterization**

Adult fish were crossed at least twice prior to phenotypic characterization. Females and males were kept in equal numbers per tank. To quantify egg quality, individual male and female fish were set up together in breeding cages the night before mating. Wild-type and mutant sibling females were crossed to wild-type male fish. Fish were put together by removing the divider and allowing fish to mate for approximately 60 min. Eggs were collected from individual breeding cages and moved to E3 medium with 0.1% methylene blue at 28 °C. Eggs were scored under a dissection microscope. Eggs were considered as ‘good quality’ if they were normal-looking one-cell stage embryos (clear separation of yolk and cell; normal egg activation and chorion swelling; non-opaque appearance). Eggs were considered as ‘poor quality’ if they were opaque, deformed, and no clear cell or chorion elevation was visible. Pictures of early embryo development were taken at 0.5, 1, 2, 3, 6 and 24 hpf on a ZEISS Stemi 508 stereo microscope with camera (2× magnification, FlyCapture2 software). To calculate the fertilization rate, the number of fertilized eggs was divided by the number of ‘good quality’ eggs. Clutch size was calculated as the sum of good quality eggs (fertilized and unfertilized). The size of the embryos was measured as area (mm²) at 6 hpf in FIJI. Successful and normal embryonic development was assessed at 6 hpf. Abnormal looking embryos were removed from the dish. Survival rate was calculated by dividing the number of larvae at 24 hpf by the number of fertilized eggs. Moreover, the numbers of normal and abnormal looking larvae were counted at 4 days post-fertilization.

**Polysome gradients**

Wild-type and mutant embryos were dechorionated with pronase (1 mg ml⁻¹). 200 dechorionated embryos per genotype were lysed in 550 µl of lysis buffer (20 mM Tris-Cl pH 7.5, 30 mM MgCl₂, 100 mM NaCl, 0.25% Igepal CA-630 (v/v), 100 µg ml⁻¹ cycloheximide, 0.5 mM dithiothreitol (DTT), and 1 mg ml⁻¹ heparin). Embryo lysates were incubated for 10 min on ice and centrifuged for 10 min at 4 °C with 21,000 g. Two-hundred microlitres of clarified lysates were loaded onto a continuous 10–50% (w/v) sucrose gradient prepared in TMS buffer (20 mM Tris-Cl pH 7.5, 140 mM NaCl). Gradients were centrifuged in a SW40 Ti rotor (Beckman) at 4 × 10⁵ rpm for 165 min. Polysones were analysed using a gradient station (BioComp) coupled to a Model Triax Flow Cell detector (FC-2). The precise location of the fractions along the UV tracing was monitored using the Gradient profiler v1.25 (BioComp) software. Polysome gradients were normalized prior to quantification for baseline differences. The area under the curve was calculated for the defined fractions. Polysome-to-monomosome ratio was calculated by dividing the value of the polysome fraction by the obtained value of the 80S fraction.

**RNA isolation**

Total RNA was isolated from ten homogenized zebrafish embryos per sample using the RNeasy Mini Kit (Qiagen). RNA concentration was measured on a spectrophotometer (DeNovix DS-11 FX+). Mutant RNA concentration was normalized to wild-type RNA concentration for each experiment.

**Analysis of translation efficiency**

To calculate translational efficiencies over the time course of embryogenesis, published Polya+ RNA-seq data (GSE32898) and ribosome profiling data (GSE46512) were pre-processed according to standard bioinformatic procedures. For ribosome profiling data (ribo-seq),
3′ adapter sequences were removed using bbd.uk from bbmap v38.26, and trimmed reads were mapped to a set of rRNAs and abundant sequences downloaded from the Illumina iGenome using bowtie2. The remaining reads were aligned to the zebrafish reference genome GRCz10 using tophat2. For RNA-seq analysis, adapter sequences were removed with bbd.uk from bbmap v38.26, and abundant reads that mapped to abundant sequences using bowtie2 were removed, similar to ribo-seq reads. Remaining reads were mapped with tophat2 to the zebrafish transcriptome using the GRCz10 genome assembly and the Ensembl 82 transcriptome release.

For ribo-seq and RNA-seq, fragments per kilobase of exon per million mapped fragments (FPKM) values for the coding sequence (CDS) were calculated for transcripts with an annotated start and stop codon. FPKM values were defined as read counts over CDS (CDS length × total read count) ÷ 106. Translation efficiencies were obtained by dividing the FPKM of pico-seq by FPKM of RNA-seq. Translational efficiency values + 0.1 were used for plotting in R 4.0.2 (1 hpf: n = 35,975; 3 hpf: n = 36,872; 6 hpf: n = 39,916; 28 hpf: n = 41,690 quantified transcripts). Statistical significance was assessed by the two-sided Wilcoxon pairwise test, and effect size was estimated by Cohen’s D (0.2, ‘negligible’; 0.5–0.8, ‘medium’; > 0.8, ‘large’).

**Sample collection for ribosome isolation**

The evening prior to the zebrafish egg or embryo collections, male and female zebrafish were separated in breeding cages. To collect mature, un-activated eggs, female zebrafish were anaesthetized in the morning using 0.01% (w/v) Tricaine (25× stock solution in dH2O, buffered to pH 7.5–7.5 with 1 M Tris pH 9.0). After being gently dried on a paper towel, the female was transferred to a dry Petri dish, and eggs were carefully expelled from the female by applying mild pressure on the fish belly with a finger and stroking from anterior to posterior. The eggs were separated from the female using a small paintbrush, and the female was transferred back to the breeding cage filled with fish water for recovery. To prevent activation, eggs were kept in sorting medium (Leibovitz’s medium, 0.5% BSA, pH 9) at room temperature. Zebrafish embryos were collected from regular matings. In the case of zebrafish embryos (1,000 embryos per sample for mass spectrometry and cryo-EM, and 5,000 embryos per sample for crosslinking–mass spectrometry), eggs were collected in Petri dishes with blue water, which consist of fish water, 0.025% (v/v) Instant Ocean salts (Aquarium Systems, 218035), and 0.0001% (v/v) methylene blue (Sigma-Aldrich, M9140). Note: embryos were incubated at 28 °C, collected at the desired time points (1 hpf and 6 hpf), and incubated with 1 mg ml⁻¹ of pronase for 5 min at room temperature for dechorination.

To obtain samples from Xenopus, egg collection and in vitro fertilization were performed following a previously described protocol. In brief, sexually matured wild-type females were primed with 50 IU of pregnant mare serum gonadotropin (PMSG, ProSpec, HOR-272) one week before the experiments. In the evening before egg collection, PMSG-primed females were injected with 500 IU of human chorionic gonadotropin (hCG, Sigma-Aldrich, CG10). Freshly laid eggs were collected from regular matings. In the case of Xenopus embryos, 5,000 embryos per sample for crosslinking–mass spectrometry), eggs were collected in Petri dishes with blue water, which consist of fish water, 0.025% (v/v) Instant Ocean salts (Aquarium Systems, 218035), and 0.0001% (v/v) methylene blue (Sigma-Aldrich, M9140). Note: embryos were incubated at 28 °C, collected at the desired time points (1 hpf and 6 hpf), and incubated with 1 mg ml⁻¹ of pronase for 5 min at room temperature for dechorination.

Ribosome isolation

Samples were washed in 2 volumes of lysis buffer, containing 20 mM HEPEs-KOH pH 7.4, 150 mM KCl, 10 mM MgCl₂, 0.5 mM DTT, RNasin (Promega), 0.25% RNaseOUT (v/v) (ThermoFisher), 0.25% SUPERaseIN (v/v) (ThermoFisher), 0.25% Igepal CA-630 (v/v), and Complete TM-EDTA-free protease inhibitor (Roche). Embryos were lysed in 2 ml (for 1,000 embryos) or 5 ml (for 5,000 embryos) of lysis buffer using a pre-cooled Dounce homogenizer. Following incubation on ice for 5 min, the supernatant was cleared by centrifugation at 21,000g for 10 min at 4 °C. Ribosome isolation was adapted from ref. 66. In brief, the clarified supernatant was loaded onto a 30% (v/v) sucrose cushion prepared in buffer A (20 mM Tris-Cl pH 7.5, 2 mM magnesium acetate and 150 mM KCl) and centrifuged at 116,000g for 5 h at 4 °C in a TL100.3 rotor (Beckman). The ribosome pellet was resuspended by orbital shaking (1 h at 4 °C) in buffer B, consisting of 20 mM Tris-Cl pH 7.5, 6 mM magnesium acetate, 150 mM KCl, 6.8% sucrose (v/v), 1 mM DTT, 0.25% RNasin Plus (v/v) (ThermoFisher), and 0.25% RNase Inhibitor (v/v) (Promega). Resuspended ribosomes were loaded onto a 15–30% (v/v) sucrose gradient prepared in buffer C (100 mM KCl, 5 mM magnesium acetate, 20 mM HEPEs-KOH pH 7.4, 100 mM potassium acetate, 5 mM magnesium acetate, and 1 mM DTT) in a TL-A100.3 rotor (Beckman) at 116,000g for 5 h. The ribosome pellet was resuspended in 120 ml of RNC buffer.

For mass spectrometry and cryo-EM, sucrose was removed from the gradient fractions by loading the resuspended ribosome solution on a Zeba Spin Desalting Columns (7K MWCO).

To analyse ribosome composition by western blot, 1,000 embryos or larvae were used per condition. Total lysate samples were collected from the cell free extract after the initial centrifugation step. The cytoplasmic fraction overlaying the sucrose cushion was collected after the ultracentrifugation step. Pelleted ribosomes after the first ultracentrifugation step were directly resuspended in 200 µl 1× Laemmli buffer. All collected fractions were frozen in liquid nitrogen.

**Digest of total cell lysates and ribosomes for mass spectrometry**

Total cell lysates and isolated ribosomes from zebrafish eggs and embryos and from Xenopus eggs were either directly denatured in 8 M urea in 100 mM ammonium bicarbonate (ABC) buffer or aceton precipitated before being dissolved in 8 M urea in 100 mM ABC. DTT was added to a final concentration of 10 mM and the sample was incubated 1 h at 37 °C. Alkylation was performed by adding iodoacetamide (IAA) to a final concentration of 20 mM and incubating for 30 min at room temperature in the dark. The reaction was quenched by addition of 5 mM DTT and incubated again 30 min at room temperature.

Samples were diluted to 6 M urea with 100 mM MAB followed by addition of Lys-C (FUJIFILM Wako Pure Chemical Corporation) at a ratio of 1:100 and incubation at 37 °C for 2 h. The samples were diluted to 2 M urea with 100 mM MAB. Trypsin (Promega, Trypsin Gold) was added at a ratio of 1:100 and incubated at 37 °C overnight. Five-hundred nanograms of each sample were analysed by liquid chromatography–mass spectrometry (LC–MS/MS).

**Crosslinking of purified ribosomes and digest for mass spectrometry**

For elution of zebrafish 1 hpf and Xenopus egg ribosome fractions used in crosslinking–mass spectrometry experiments, buffer C was adjusted to 20 mM HEPEs-KOH pH 7.6, 100 mM KCl, and 5 mM magnesium acetate. 0.5 mM of DSSO from a 5 mM stock solution (in dimethyl sulfoxide (DMSO)) was added to 10 µl of 500 nM ribosomes and incubated for 45 min at room temperature. The reaction was quenched by adding Tris-Cl pH 7.5 to a final concentration of 100 mM and incubated for 15 min at room temperature. The band pattern of the crosslinking reaction was analysed by SDS–PAGE.
DSSO-crosslinked ribosomes were denatured in 8 M urea, 100 mM ABC followed by reduction, alkylation and proteolytic digest using Lys-C and trypsin (both added 1:20) as described above. Digests were acidified to 1% trifluoroacetic acid (TFA) and desalted using Oasis HLB Sorbent (Oasis HLB Microelution plate, Waters) according to the manufacturer’s description. Peptides were eluted with 2 × 30 µl 70% acetonitrile (ACN), 0.1% TFA and the sample was concentrated under reduced pressure to 20 µl. The samples were supplemented with 5% DMSO. To enrich for crosslinked peptides, the samples were fractionated by size-exclusion chromatography (SEC) on a TSKgel SuperSW2000 column (300 mm × 4.5 mm × 4 µm, Tosoh Bioscience), which was operated at 200 µl min⁻¹ in 30% ACN, 0.1% TFA. Fractions were collected every minute, and ACN was removed under reduced pressure. DMSO was again added to 5%.

LC-MS/MS

Generated peptides were analysed on a nano-reversed phase HPLC (RSLC nano system, ThermoFisher) coupled to a Q Exactive HF-X mass spectrometer (ThermoFisher), equipped with a Nanospray Flex ion source (ThermoFisher). Peptides were loaded onto a trap column (PepMap C18, 5 mm × 300 µm ID, 5 µm particles, 100 Å pore size) at a flow rate of 25 µl min⁻¹ using 0.1% TFA as mobile phase. After 10 min, the trap column was switched in line with the analytical column (PepMap C18, 500 mm × 75 µm ID, 2 µm, 100 Å, ThermoFisher), which was operated at a flow rate of 230 nl min⁻¹ at 30 °C. For separation a solvent gradient was applied, starting with 98% buffer A (0.1% formic acid in water) and 2% buffer B (80% ACN, 0.1% formic acid), followed by an increase to 35% buffer B over the next 180 or 360 min for digested lysates and ribosomes. For unfractionated and SEC-enriched crosslinked samples, a gradient to 40% buffer B in 180 or 120 min, respectively, was used. This was followed by a steep gradient to 90% buffer B in 5 min, staying there for five min and decreasing to 2% buffer B in another 5 min.

For the digested lysates and ribosomes, the mass spectrometer was operated in data-dependent mode, using a full scan (m/z range 375–1500, resolution of 60,000, target value 10⁶) followed by MS/MS scans of the 10 most abundant ions. MS/MS spectra were acquired using a normalized collision energy of 28, isolation width of 1.4 m/z, resolution of 30,000, maximum fill time of 105 ms and a target value of 10⁶. Precursor ions selected for fragmentation (excluding charge state 1, 7, 8, >8) were put on a dynamic exclusion list for 60 s. Additionally, the minimum AGC target was set to 5 × 10⁴ and intensity threshold was calculated to be 4.8 × 10⁴. The peptide match feature was set to preferred, and the exclude isotopes feature was enabled.

For crosslinked samples, the mass spectrometer was operated in data-dependent mode, using a full scan (m/z range 350–1,600, resolution of 120,000 and a target value of 10⁹) followed by MS/MS scans of the 15 most abundant ions. MS/MS spectra were acquired using a stepped normalized collision energy of 27 ± 6, isolation width of 1 m/z, resolution of 30,000, maximum fill time of 150 ms and a target value of 5 × 10⁴. Precursor ions selected for fragmentation (excluding charge state 1, 2, >7) were put on a dynamic exclusion list for 30 s. Additionally, the minimum AGC target was set to 5 × 10⁴, and intensity threshold was calculated to be 3.3 × 10⁴. The peptide match feature was set to preferred, and the exclude isotopes feature was enabled.

Analysis of mass spectrometry data

For peptide identification, the RAW files were loaded into Proteome Discoverer (v2.5.0.402, ThermoFisher). All hereby created MS/MS spectra were searched using MS Amandap v2.5.0.16129, Engine v2.0.0.16129. For the first step search, the RAW files were searched against a combined zebrafish sequence database (Danio rerio GRCh21) and Uniprot downloaded on 21 March 2019 (58,524 sequences; 34,079,443 residues), and against the Xenopus laevis Uniprot proteome UP000186698. All RAW files were supplemented with common contaminants using the following search parameters: (1) the peptide mass tolerance was set to ±5 ppm and the fragment mass tolerance to ±0.05 ppm, and (2) the maximal number of missed cleavages was set to 2 using tryptic enzymatic specificity. The result was filtered to 1% FDR on protein level using Percolator algorithm⁶⁸ integrated in Proteome Discoverer. A sub-database was generated for further processing. For the second step, the RAW files were searched against the created sub-database. Oxidation on methionine, deamidation on asparagine and glutamine, phosphorylation on serine, threonine and tyrosine; iodoacetamide derivative on cysteine, beta-methylthiolation on cysteine and carbamylation on lysine were set as variable modifications. Monoisotopic masses were searched within unrestricted protein masses for tryptic enzymatic specificity. The peptide mass tolerance was set to ±5 ppm and the fragment mass tolerance to ±0.05 ppm. The maximal number of missed cleavages was set to 2. The result was filtered to 1% FDR on peptide level using the Percolator algorithm integrated in Proteome Discoverer. The localization of the post-translational modification sites within the peptides was performed with the tool ptmRS, based on the tool phosphoRS⁶⁹. Peptide areas were quantified using the in-house-developed tool apQuan⁷⁰. Proteins were quantified by summing unique and razor peptides and applying the iBAQ calculation. Normalization of protein abundances was performed on the sum of areas of all identified ribosomal proteins. Statistical significance of differentially expressed proteins was determined using limma⁷¹.

Analysis of crosslinking-mass spectrometry data

Data analysis was performed within Proteome Discoverer v2.5.0.402 using MS Amandap (v1.0.18345)⁷₂. The workflow tree consisted of the MS Amandap Detector node (mass spectrometry tolerance 10 ppm, crosslink modification: DSSO +185.004 Da at lysine and at protein N termini, diagnostic ions: 138.0911; 155.179; 170.0634; 187.0900, crosslink modification addition: 18.010565, doublet pair selection in combined mode) followed by MS Amandap Search (full tryptic digest, 5/10 ppm peptide/fragment mass tolerance, maximal 3 missed cleavages, carba- methylidonyl +57.021 Da at cysteine as static and oxidation +15.995 Da at methionine as dynamic modification) and completed with MS Amandap Validator (1% FDR cutoff at CSM and crosslink level, separate Intra/Inter-link FDR set to false). The search was performed against a database generated from a mass spectrometry analysis of a digested non-crosslinked zebrafish (containing 1,092 proteins) or Xenopus (containing 1,173 proteins) ribosome sample, respectively. To generate these specific databases the two non-crosslinked samples were searched using MS Amandap against the zebrafish Uniprot reference proteome UP000000437 (downloaded 2020-09-15, 46,847 sequences, 24,556,292 residues) or X. laevis Uniprot proteome UP000186698 (downloaded 2020-01-07, 43,235 sequences, 19,251,436 residues), both supplemented with common contaminants. Search parameters were set as described for time course experiments but using 5/10 ppm peptide/fragment mass tolerance and maximum 3 missed cleavages. Carbamidomethylation on cysteine was set as a fixed modification, oxidation on methionine, deamidation on asparagine and glutamine and acetylation on the protein N terminus were set as variable modifications.

To validate the quality of the chosen search strategy, an additional crosslink search was performed using equal settings but a database file containing sequences aligned to the dormant ribosome structures of Xenopus and zebrafish. The resulting crosslinks at 1% FDR were plotted onto the structure using ChimeraX v1.1⁷³, and the distance between connected amino acids (Ca–Ca distance) was measured. Crosslinks including Ca residues missing in the structure file were removed, yielding 360 and 178 crosslinks aligned to the zebrafish and Xenopus structures, respectively.

DNA constructs and mRNA synthesis

dap, dap1b, habp4, Renilla luciferase and zebrafish β-globin constructs and dap/dap1b mutant and chimeric versions for in vitro translation in
Article

**Recombinant protein expression and purifications**

Zebrafish dap1b and dap coding sequences were codon optimized for *Escherichia coli*, cloned into a vector providing an N-terminal 6×His tag followed by a 3C protease-cleavage site, and transformed into BL21(DE3) E. coli cells. For Dap1b expression, cells were grown in LB at 37 °C until reaching an A600 of 0.7, induced with 0.5 mM IPTG, and grown overnight at 18 °C. For Dap expression, bacterial cultures were grown on terrific broth medium supplemented with 1.5% (w/v) lactose for 1 h at 37 °C, and for 23 h at 18 °C. Cells were pelleted, resuspended in lysis buffer (50 mM Tris-Cl pH 8.0, 300 mM NaCl, Complete Protease Inhibitor Cocktail (Merck)) and lysed by sonication (19 mm probe, 4 min: pulse on 1 s, pulse off 2 s, 60% amplitude). The intracellular soluble fraction was isolated by centrifugation at 20,000 xg, 30 min, 4 °C. Imidazole was added to a final concentration of 20 mM. The lysate was loaded onto a 5 ml His Trap FF column (Merck), washed with buffer A (50 mM Tris-Cl pH 8.0, 300 mM NaCl), and eluted with buffer A supplemented with 500 mM imidazole. 1 mg of 3C protease was added to the elution fraction during dialysis overnight against buffer A. The cleaved protein was loaded onto a 5 ml His Trap FF column (Merck); the flow-through, containing the cleaved protein, was concentrated (Vivaspin 20, MWCO 5 kDa) and loaded onto a Superdex 75 26/60 column (GE Life Sciences) equilibrated with SEC buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl).

**Analysis of de novo translation**

De novo translation in zebrafish embryos was measured using incorporation of puromycin. One-hundred eighty micromolar puromycin (approximate final concentration 2 μM) was injected into 1-cell stage embryos. Embryos were incubated for 30 min, during which they were manually dechorionated. Following the 30 min incubation period, embryos were manually deyolked (de-capped). Cell caps were immediately transferred into 1× Laemmli sample buffer and snap-frozen in liquid nitrogen. Each individual replicate consisted of embryos derived from a single pair of wild-type fish and a single pair of mutant fish of the respective genotype. Only two pairs (one wild-type and one mutant pair) were allowed to mate at the same time, and the exact time point of the mating onset was recorded for each pair and sample processing was adapted accordingly. For each sample 10 embryos were lysed in 50 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA containing Complete Protease Inhibitor Cocktail (Roche, 11836170001). Embryos treated with 100 μg ml−1 cycloheximide 10 min prior to injection with puromycin were used as a negative control. Puromycin incorporation was assessed by western blotting.

**In vitro translation assays**

RRL (Green Hectares) were cloned into pCDNA3.1 by Gibson assembly

Zebrafish dap1b and dap coding sequences were codon optimized for *Escherichia coli*, cloned into a vector providing an N-terminal 6×His tag followed by a 3C protease-cleavage site, and transformed into BL21(DE3) E. coli cells. For Dap1b expression, cells were grown in LB at 37 °C until reaching an A600 of 0.7, induced with 0.5 mM IPTG, and grown overnight at 18 °C. For Dap expression, bacterial cultures were grown on terrific broth medium supplemented with 1.5% (w/v) lactose for 1 h at 37 °C, and for 23 h at 18 °C. Cells were pelleted, resuspended in lysis buffer (50 mM Tris-Cl pH 8.0, 300 mM NaCl, Complete Protease Inhibitor Cocktail (Merck)) and lysed by sonication (19 mm probe, 4 min: pulse on 1 s, pulse off 2 s, 60% amplitude). The intracellular soluble fraction was isolated by centrifugation at 20,000 xg, 30 min, 4 °C. Imidazole was added to a final concentration of 20 mM. The lysate was loaded onto a 5 ml His Trap FF column (Merck), washed with buffer A (50 mM Tris-Cl pH 8.0, 300 mM NaCl), and eluted with buffer A supplemented with 500 mM imidazole. 1 mg of 3C protease was added to the elution fraction during dialysis overnight against buffer A. The cleaved protein was loaded onto a 5 ml His Trap FF column (Merck); the flow-through, containing the cleaved protein, was concentrated (Vivaspin 20, MWCO 5 kDa) and loaded onto a Superdex 75 26/60 column (GE Life Sciences) equilibrated with SEC buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl).

Western blotting was performed following standard protocols. In brief, protein samples were boiled for 5 min in 1× Laemmli sample buffer and separated by SDS–PAGE using Mini-PROTEAN TGXTM Precast Protein Gels or 10–20% Mini-PROTEAN Tris-Tricine Gels (Bio-Rad). Blotting was performed onto a nitrocellulose membrane (GE Healthcare) using a wet blot system (Bio-Rad). The following antibodies were used: anti-Flag (mouse, 1:1,000, Sigma-Aldrich F1804), anti-eEF2 (rabbit, 1:1,000, Proteintechn, 20107-1-AP), anti-puromycin (mouse, 1:20,000, Sigma-Aldrich, MAE343), anti-α-tubulin (mouse, 1:1,000, Sigma-Aldrich, T6074), anti-RL3 (rabbit, 1:1,000, GeneTex, GTX124464), F(ab’)2 anti-rabbit IgG (H+L)-HRPO (goat, 1:10,000, Sigma-Aldrich, 116,000), F(ab’)2 anti-mouse IgG (H+L)-HRPO (goat, 1:10,000, 111-036-045, Dianova), and F(ab’)2 anti-mouse IgG (H+L)-HRPO (goat, 1:10,000, 116,000). The chemiluminescent signal was quantified using ImageJ 1.80_172. To quantify ribosome binding of Flag-tagged proteins after in vitro translation, signal intensities were simultaneously measured for Flag, RPL3 and eEF2 for all three membranes (total reaction, supernatant, ribosome pellet). The ratio between the Flag signal in the supernatant and the ribosome pellet was normalized to the total amount translated (Flag signal in the total reaction) and to sample loading (RPL3 and eEF2 signals). To quantify de novo translation in wild-type and mutant embryos, signal intensities were measured for puromycin, RPL3 and α-tubulin. The puromycin signal was normalized to the RPL3 (per ribosomal content) or α-tubulin (per embryo) signal. Transgenic expression of tagged Dap and Dap1b as well as changes in ribosome binding during development were determined by measuring the signal intensity for Flag, RPL3, α-tubulin and eEF2 for all three membranes (total reaction, supernatant, ribosome pellet). The relative amount in each fraction was determined by normalization of the Flag signal in the supernatant and in the ribosome pellet fraction to the total amount expressed at the respective stage and the amount loaded. Raw images of uncropped blots are shown in Supplementary Fig. 2.

Grid preparation for cryo-EM

A 2 nm thick continuous carbon film was produced on an Auto306 high vacuum evaporator (Boc Edwards). This film was floated onto Quantifoil Cu 3.5/1, 200 mesh grids, and grids were dried. Grids were glow-discharged for 1 min in a SCD005 sputter coater (Bal-Tec) at -20 mA. Four microlitres of sample (at a concentration of 200 ng μl−1 of RNA, which corresponds to about 100 nM ribosomes) were applied to the grid and incubated for 60 s at 70% humidity and 4 °C in a Leica EM GP. Subsequently, grids were blotted for 2 s using the proximity sensor and plunge-frozen in liquid ethane at −180 °C.

Cryo-EM data collection

All grids were screened on a Glacios TEM (ThermoFisher) to check for particle distribution and grid quality; data were recorded with EPU2.
Grids that passed the evaluation were recorded on a Titan Krios microscope equipped either with a Falcon 3EC or a K3 detector, using the software SerialEM v3.8. A pixel size of about 1 Å per pixel was chosen, and micrographs with a total dose of about 40 e⁻ Å⁻² fractionated in 39 frames were collected with a target defocus between −1 and −2.5 μm.

**Electron microscopy data processing**

Most data processing was performed in Cryosparc version 2.16.0β. Micrographs were motion corrected and dose weighted using Patch motion correction. Contrast transfer function (CTF) parameters were determined using Patch CTF. About 500 particles were manually selected and 2D classified to create templates. Automated particle picking was performed using these templates. Picking was manually inspected, and a threshold that excludes low signal-to-noise and high signal-to-dirt false positive picks and minimizes the false negative picks was chosen. The remaining particles were extracted with a box size between 440 and 512 pixels and subjected to 2D classification. Classes that showed clear ribosomal densities were selected and subjected to heterogeneous ab initio model generation. Subsequently, the remaining particles were 3D refined to generate a consensus model. Afterwards, optics groups were assigned to the individual particles depending on the applied beam shift during recording. Global and local CTF refinements were then performed, and all particles were refined using non-uniform refinement. To generate masks for local refinement, the density was filtered to 20 Å, and sub-densities of the entire 40S subunit and the head region were generated using the Volume eraser tool in UCSF Chimera 1.13.1β. These densities were binarized, extended by 7 pixels and another 7 pixels as soft edge. Locally refined maps using the corresponding masks were obtained in Cryosparc, and composite maps were generated with the vop maximum command in UCSF Chimera 1.13.1β.

To analyse particle dynamics and heterogeneity, the consensus refined particles were subjected to CryoDRGN analysis. Due to the large pixel box size of our datasets, the dimensions of the ribosome particles were reduced to allow for feasible computation. The particles were down-sampled to a box size of 128 or 256 pixels, and two iterative rounds of the CryoDRGN VAE were trained using a network with 3 decoder and 3 encoder layers with 1,024 as outer dimensions and a bottleneck latent dimension of 8. The resulting z values were classified using k-means clustering and Gaussian mixture models and plotted using a UMAP approach. Particles belonging to class 1 (containing all four factors) from the Xenopus egg ribosome dataset were selected from the originally refined particles in CryoDRGN’s Jupyter notebook and subjected to an additional round of 3D refinement. Only particles located at the centre of the cluster were taken in order to obtain a map as free from heterogeneity as possible. The final maps were polished with deepEMhancerβ.

**Model building and refinement**

The molecular models of the 1 hpf and 6 hpf ribosomes from zebrafish were built using PDB 4UG0 as an initial model for core ribosomal proteins and rRNA. In the case of the 1 hpf ribosome, additional factors were modelled from previously published structures: eIF5A from PDB 5DAT, and eEF2 and Hapb4 (SERBP1 parologue) from PDB 6MTE. All chains were first rigid body-fitted in Coot 0.8.979 and manually mutated to the corresponding sequences in Xenopus, taking into consideration the mass spectrometry data from purified Xenopus ribosomes. Dapl.S was built de novo in Coot and then used as a template to build Daplb in the zebrafish 1 hpf ribosome. Although two Dap proteins (Dapl.S and Dapll in Xenopus, and Daplb and Dapb in zebrafish) could fit into the corresponding densities, Dapl.S and Dapb were modelled according to gene expression data (Extended Data Fig. 5b–d) and protein abundance (measured by mass spectrometry; Fig. If and Extended Data Fig. 1a).

The rabbit ribosome model with recombinant zebrafish Daplb was built using 6MTE as an initial model for core ribosomal proteins, rRNA, eEF2 and SERBP1. eIF5A was modelled using PDB 5DAT as reference. All models were manually adjusted to fit the observed density in Coot 0.8.9.β and real-space-refined using Phenix 1.7.1β. The 40S subunit of the rabbit ribosome was only submitted to rigid body fitting in Phenix. Working files were saved in PDB and the final models were converted to mmCIF using PDB_extractβ. Cryo-EM densities and models were visualized using UCSF ChimeraX version 0.9.1β. Structure alignments were performed using the command mmaker in ChimeraX.

**Previously published data**

Previously published structures used in this study are PDB IVY4, PDB 3JCT, PDB 4UG0, PDB 5DAT, PDB 5GAK, PDB 5HAU, PDB 5O2R, PDB 6HCF, PDB 6MTE, PDB 6OLE, PDB 6SGC, PDB 6RM3 and PDB 6RRZ. The ribosome profiling data and RNA-seq data were published previously and are accessible at Gene Expression Omnibus (GEO) with accession numbers GSE46512β (ribosome profiling), GSE32900β (subseries GSE32898) and GSE147112 (RNA-seq).

**Reporting summary**

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

**Data availability**

Cryo-EM maps and molecular models generated in this study have been deposited in the Protein Data Bank (PDB) under accessions 7OYA (zebrafish 1 hpf), 7OYB (zebrafish 6 hpf), 7OYC (Xenopus eggs) and 7OYD (rabbit ribosome with zebrafish Daplb), and in the Electron Microscopy Data Bank with accession codes EMD-13111 (zebrafish 1 hpf), EMD-13112 (zebrafish 6 hpf), EMD-13113 (Xenopus egg ribosome) and EMD-13114 (rabbit ribosome with zebrafish Daplb). Raw micrographs, particle stacks and CryoDRGN models were deposited at the EMPIAR Database (EMPIAR-11274). Mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDEβ partner repository with the dataset identifier PXD026866. Source data are provided with this paper.

**Code availability**

No custom codes were used for this study.

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Author contributions F.L., L.L.-O., D.H. and A.P. conceived the study. F.L. performed most experiments with the help of C.P., further supported by J.R. and A.C. L.L.-O. obtained the final cryo-EM maps and modelled all ribosomes with contributions of F.L., A.M. and D.H. and help from S.K. I.G. prepared and screened grids, collected EM data, and together with F.L. and D.H., contributed to the initial cryo-EM data processing with help from K.B. M.M. and E.R. performed and analysed the crosslinking sample preparation, and K.M. supervised mass spectrometry experiments and analysed the crosslinking samples. T.Y.L. provided Xenopus eggs and 24 hpf embryos. D.H. performed the cryo-RiGN analysis and F.L. and L.L.-O. compared the resulting maps. A.P. and D.H. supervised the project. L.L.-O., F.L. and A.P. wrote the manuscript with input from all authors.

Competing interests The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Mass-spectrometry and cryo-EM analysis of *Xenopus* egg ribosomes. a, Fold change of ribosome-associated factors and core ribosomal proteins after comparing mass spectrometry data of purified ribosomes from unfertilized *Xenopus* eggs and 24 hpf larvae (stage 14) (n = 1 experiment). b, Processing pipeline of the *Xenopus* egg ribosome. All steps were done in Cryosparc v3.2.0. Maps are shown in grey, masks in blue. c–d, Maps showing the local resolution of Map1 and Map2 (c), and of the four factors associated with *Xenopus* egg ribosomes (d). Note that the resolution of the large subunit in Map2 (in c) is 0 (shown in blue) since this region was outside the mask used for obtaining this map (see b). e, Orientation distribution plot for all particles contributing to Map1. f, Gold-Standard Fourier Shell Correlation (GSFSC) of Map1.
Extended Data Fig. 2 | Processing pipeline of the 1 hpf zebrafish ribosome. All steps were done in Cryosparc v3.2.0. Maps are shown in grey, masks in blue. The orientation distribution plot for all particles contributing to Map1 and the Gold-Standard Fourier Shell Correlation (GSFSC) of the respective map is shown on the bottom. Local resolution maps were calculated for Map1, Map2, Map3, and for the four ribosome-associated factors. Note that in the box with local resolution maps, the resolution of the large subunit in Map2 and Map3, and of the small subunit’s head in Map3 is 0 (shown in blue) since these regions were outside the masks used for generating these maps.
Extended Data Fig. 3 | Characterization of the dormant ribosome state in zebrafish 1 hpf and Xenopus egg ribosomes.  

**a–d.** Densities of the two modules, namely Habp4•eEF2b/eEF2 (a–b), and Dap1b/Dap1l•eIF5a (c–d), that are characteristic for dormant ribosomes in zebrafish 1 hpf embryos and Xenopus eggs. 

**e.** Overview of the ribosome structure isolated from 6 hpf zebrafish embryos lacking the specific egg ribosome-associated factors.

**f.** Latent space representations of ribosomal particles from Xenopus eggs (left), 1 hpf zebrafish embryos (middle) and 6 hpf zebrafish embryos (right) as UMAP embeddings after training a cryoDRGN latent variable model. Classes are depicted in Roman numbers, map volumes are indicated with Arabic numbers. Total particle numbers are shown on the top left of each graph. 

**g.** Densities of ribosome-associated factors from Xenopus eggs. An overview of the map is shown on the top left.

**h.** Densities of ribosome-associated factors in a map reconstructed from class I particles from Xenopus eggs obtained with cryoDRGN. An overview of the map is shown on the top-left.
Extended Data Fig. 4 | Processing pipeline of the 6 hpf zebrafish ribosome. All steps were done in Cryosparc v3.2.0. Maps are shown in grey, masks in blue. The orientation distribution plot for all particles contributing to Map1 and the Gold-Standard Fourier Shell Correlation (GSFSC) of the respective map is shown on the bottom-right. Local resolution maps were calculated for Map1, Map2 and Map3. Note that in the box with local resolution maps, the resolution of the large subunit in Map2 and Map3, and of the small subunit’s body in Map3 is 0 (shown in blue) since these regions were outside the masks used for generating these maps.
Extended Data Fig. 5 | Sequence conservation of the Dap/Dap1b/Dapl1 protein family and RNA expression of ribosome-associated factors.

a, Protein sequence alignment of the Dap/Dap1b/Dapl1 protein family illustrates conserved motifs. Vertebrates have two paralogs, namely Dap1b/Dapl1 and Dap. Invertebrates only encode one homolog (Dap1) that clusters in between Dap1b/Dapl1 and Dap proteins.

b, Zebrafish mRNA expression levels (PolyA+ RNA-seq31,82) of eif5a/eif5a2 (purple), eef2b (orange), dap1b/dap (green) and habp4 (blue) during oogenesis and embryogenesis.

c, Xenopus mRNA expression levels of all paralogs of eif5a, eef2, dap, dap1b and habp4 derived from riboMinus-seq data84. d, mRNA expression levels of zebrafish eif5a/eif5a2, eef2b, dap1b/dap and habp4 in adult tissues82. TPM, transcripts per million.
Extended Data Fig. 6 | Structural comparison of Dap1b and other factors that insert into the polypeptide exit tunnel (PET). a, Structures of ribosomes with proteins and peptides inserted into the PET. From left to right: zebrafish Dap1b inserted into the rabbit ribosome, Bac7 (5HAU), Api137 (5O2R), Rei1 (6RZZ), Nog1 (3JCT) and MDF2 (6RM3). Models were clipped to have a better view of the PET. Boxed areas (dashed boxes) are shown at higher magnification in b. b, Detail of the peptidyl-transferase center (PTC) of the ribosomes shown in a. The dashed line indicates the position of the PTC. All previously known factors use different mechanisms than Dap1b to achieve their functions: Bac7 interacts with the ribosomal A-loop via its N terminus (see below) to block translation initiation, Api137 interacts with the release factors RF1 or RF2 to block termination of bacterial ribosomes, Rei1 and Nog1 insert into the PET of 60S subunits during ribosome biogenesis and their C termini do not extend beyond the PTC, and MDF2’s C terminus interferes with P-tRNA and elF5A binding to establish dormancy in the microsporidian ribosome.

c, Superimposition of Dap1b (left) and Bac7 (right) with eukaryotic (5GAK) and prokaryotic (1VY4) A- and P-tRNAs, respectively. Red asterisks denote clashes of Dap1b and Bac7 with the A-tRNA.

d, Dap1b’s C terminus does not interact with the A-loop (right), in contrast to Bac7 (left). Dashed lines mark distances between Arg1 of Bac7 and Phe109 of Dap1b with a conserved uracil of the A-loop.

e, Scheme of the interactions of Dap1b’s C terminus within the ribosome. Dap1b interacts with helix 74 (H74) and H90 of the 28S rRNA.

f, Scheme of the interactions of Bac7’s N terminus within the ribosome. Bac7 interacts with H89 and the A-loop (H92).
Extended Data Fig. 7 | Crosslinking and mass spectrometry (MS) analysis of ribosomes from 1 hpf zebrafish embryos and *Xenopus* eggs. a, Cα-Cα distance distribution of the DSSO-induced crosslinks identified in *Xenopus* egg and zebrafish 1 hpf ribosomes. b–c, Proteins crosslinked to zebrafish Habp4 (b) or Dap (c) are shown on the 1 hpf zebrafish ribosome as surface representations, with crosslinked residues depicted in darker color. d, Crosslinking mapping of Habp4 (shown as a scheme; modeled regions highlighted with a black line) to proteins of the zebrafish embryo (top) and *Xenopus* egg (bottom) ribosome. Crosslinked proteins are shown as surface representations, with crosslinked residues depicted in dark blue. A cartoon (right) shows a model of Habp4 on the ribosome. e, Crosslinking mapping of zebrafish Dap (top) and *Xenopus* Dapl1 (bottom; for details, see d).
Extended Data Fig. 8 | Characterization of single (habp4−/−), double, and triple (dap−/− dap1b−/− habp4−/−) zebrafish mutants. a–b, Early embryo development of habp4−/− (a) and dap−/− dap1b−/− mutants (b) compared to wildtype (WT). c, Mendelian ratio analysis of fin-clips from adult fish obtained from heterozygous habp4+/− parents (25% expected to be habp4−/−; n = 4 for both genotypes). d, Size of WT and habp4−/− embryos at 6 h post-fertilization (hpf) (WT: n = 31; WT-2: n = 22; WT-3: n = 35; habp4−/−: n = 36; habp4−/−−2: n = 24; habp4−/−−3: n = 33). e, Total RNA of 1–3 hpf embryos (WT: n = 42; dap−/− dap1b−/−: n = 41; WT and triple KO: n = 20 for both genotypes). f, Number of eggs laid by single, double, and triple KO females compared to matching WT. g, Representative images (left) and quantification (right) of poor quality eggs. h, Percentage of embryos from single, double, and triple KO mutant pairs displaying normal embryo development until 6 hpf compared to matching WT pairs. i–j, Relative number of embryos and larvae (in relation to the embryos that developed normally up to 6 hpf; see h) that showed abnormalities at 1 (i) and 4 (j) days post-fertilization (dpf). Example images are shown on the left. In c, e, i, and j, data are presented as scatter dot plots with means ± standard deviation (SD). Data in d are represented as a violin plot with median and quartiles. In h, dotted vertical lines indicate separate experiments. Significance was determined using Kruskal-Wallis and Dunn’s two-sided test (d, f, j) for more than 2 sample group comparisons or Mann-Whitney test (c, e, and i, h) for pairwise comparisons between habp4−/− or dap−/− dap1b−/− versus WT. For c and f, n are independent crosses; for d, n are individual embryos; for e, n are biologically independent samples. #, number of crosses.
Extended Data Fig. 9 | Recombinant Dap1b binds to the polypeptide exit tunnel of rabbit ribosomes. a, b, Western blot of the total in vitro translation reaction shown in Fig. 4c (a) and Fig. 4e (b). Uncropped images of membranes are provided in Supplementary Fig. 2a, b. c, Translation activity assays (Fig. 4a top) of renilla luciferase mRNA upon addition of increasing concentrations of C-terminal Dap and Dap1b peptides. BSA and N-terminal Bac7 are used as negative and positive controls, respectively (n = 4 biologically independent samples). Dots represent means and error bars are standard deviation (SD). d, Ribosome binding assays (Fig. 4a bottom) of in vitro translated FLAG-tagged Dap-Dap1b chimeras compared to full-length (WT) Dap and Dap1b. A representative Western blot from a single experiment is shown on the left; quantification of three independent experiments is shown on the right. Data are represented as scatter dot plots with means ± standard deviation (SD). Significance was assessed with Kruskal-Wallis followed by Dunn’s two-sided test. Uncropped images of membranes are provided in Supplementary Fig. 2d. e, Mass spectrometry data of ribosomes isolated from habp4−/− and WT (left), and from dapi−/− dap1b−/− and WT (right) embryos at 1 hpf, represented as volcano plots (n = 3 independent experiments). Permutation-based false discovery rates (FDRs) are displayed as dotted (FDR < 0.01) and dashed (FDR < 0.05) lines.
Extended Data Fig. 10 | Processing pipeline of the rabbit ribosome with recombinant zebrafish Dap1b. a, Processing pipeline for obtaining an 80S density map of the rabbit ribosome with zebrafish Dap1b. b, Orientation distribution plot (top) and Gold-Standard Fourier Shell Correlation (GSFSC; bottom) of Map1. c, Local resolution maps calculated for Map2 and Map3. Densities and local resolutions of eIF5A, Dap1b, eEF2 and SERBP1 are shown on the bottom. Note that the resolution of the small subunit in Map2 is 0 (shown in blue) since this region was outside the mask used for generating this map. d, Latent space representation of particles from rabbit ribosomes with zebrafish Dap1b as a UMAP embedding after training a cryoDRGN latent variable model. Classes are depicted in Roman numbers, map volumes are indicated with Arabic numbers. Total particle number is shown on the top left of the graph.
Reporting Summary

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 Software and code

Policy information about availability of computer code

Data collection

Cryo-EM data from grids screened on a Glacios TEM (ThermoFisher Scientific) were recorded with EPU 2. Grids that past the initial screen were then recorded on a Titan Krios Microscope, using the software SerialEM v3.8. Absorbance at 260 nm of polysome gradients were recorded with Gradient profiler v1.25 (BioComp). Renilla luciferase luminescence data were obtained using the software Gen5 (BioTek Instruments, Inc.). Peptides obtained by mass spectrometry (MS) and crosslinking-MS were identified using the software Proteome Discoverer (v2.5.0.402,631 ThermoFisher Scientific). Pictures of early embryo development were taken on a ZEISS Stemi 508 stereo microscope with camera [FlyCapture2 software].

Data analysis

Cryo-EM data were analyzed using Cryosparc v2.16.0, UCSF Chimera 1.13.1, cryoDRGN (Zhong et al. 2021) and DeeplMhancer (Sanchez-Garcia et al. 2021). Ribosome structures were modeled and refined using Coot 0.8.9 and Phenix 1.17.1. Files were saved in PDB and the final models were converted to mmCIF using PDB_extract [https://pdb-extract.wwpdb.org/]. Densities and models were visualized using UCSF ChimeraX 0.91.

Ribosome profiling data were analyzed using Bbduk from Bbmap v38.26, Bowtie2 and Tophat2. RNA-seq data were analyzed using Bbduk from Bbmap v38.26 and Bowtie2 in R R (version 4.0.2).

MS spectra was analyzed with Proteome Discoverer (v2.5.0.402,631 ThermoFisher Scientific), using MS Amanda v2.5.0.16129 and Engine v2.0.0.16129. Peptide areas have been quantified using apQuant (Deblmann et al. 2019) and statistical significance was determined with limma (Smyth, 2005). Crosslinking-MS data analysis was performed with Proteome Discoverer v2.5.0.402, using MS Annika v1.0.18345, MS Amanda v2.5.0.16127 and ChimeraX v1.1.

Most graphs and statistical analysis were generated with GraphPad Prism v8.0.2 or R (version 4.0.2).

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Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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Cryo-EM maps and molecular models generated in this study have been deposited in the Protein Data Bank (PDB) with IDs 7OYA [zebrafish 1 hpf], 7OYB [zebrafish 6 hpf], 7OYC [Xenopus egg] and 7OYG [rabbit ribosome with zebrafish Dap15]. And in the Electron Microscopy Data Bank with accession codes EMD-13111 [zebrafish 1 hpf], EMD-13112 [zebrafish 6 hpf], EMD-13113 [Xenopus egg ribosome] and EMD-13114 [rabbit ribosome with zebrafish Dap15]. Raw micrographs, particle stacks and CryoEM maps models were deposited at the EMBL-EBI Data Bank (EMBL-EBI 11274). Mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE82 partner repository with the dataset identifier PXD026866. Source data are provided with this paper. Previously published structures used in this study are PDB-3VY4, PDB-3JCT, PDB-4LUG, PDB-5DAT, PDB-5GAK, PDB-5HAL, PDB-5QZ, PDB-5OQ2R, PDB-6HCF, PDB-6MTE, PDB-6OLE, PDB-6GC, PDB-6RM3 and PDB-6RZ4. The ribosome profiling data and RNA-seq data were published previously and are accessible at Gene Expression Omnibus [GEO] with accession numbers GSE4651230 (ribosome profiling), GSE3290031 (subseries GSE3289B) and GSE14771283 (RNA-seq).

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Sample size
Sample sizes are described in the figure legends. No statistical calculations were done to predetermine the sample size. Sample sizes were chosen as large as possible while still feasible in terms of sample handling and data collection.

Data exclusions
During the cryoEM analysis, false positive particle picks were excluded after careful 2D and 3D classification. This includes contaminating ice, non ribosomal particles and images with insufficient optical quality.

Replication
Replication of experimental data was successful; experiments were performed independently at least twice, with the exception of cryo-EM and MS experiments due to the efforts required in acquiring and processing these datasets. The number of independent replicates are specified in the Figure legend for each Figure panel.

Randomization
Zebrafish were grouped based on their genotypes (wildtype versus mutant) and age. In case of the analysis of mutants compared to wildtype, siblings (progeny of the same parents) were analysed within one experiment wherever possible. If this was not possible, embryos or eggs derived from parents of similar ages were used for the experiment. Both in vivo and in vitro samples were allocated randomly to the experiment and treated equally.

Blinding
Blinding was not possible during data collection since, according to our animal protocols, fish and embryos need to be correctly labeled with their genotype and date of birth. However, after sample collection, all samples (e.g. WT vs mutant) were treated equally and processed at the same time to minimize possible biases introduced during unequal sample preparations. Data analysis was performed with standard pipelines that did not include 'subjective' criteria that could influence the result.

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Methods

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|-----|-----------------------|
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| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |
Antibodies

Primary antibodies: anti-FLAG (mouse, 1:1000, Sigma-Aldrich F1804, clone M2), anti-eEF2 (rabbit, 1:1000, Proteintech, 20107-1-AP), anti-RPL3 (rabbit, 1:1000, GeneTex, GTX124464), anti-Puromycin (mouse, 1:20000, Sigma Aldrich, MABE343, clone 12D10), and anti-alpha-Tubulin (mouse, 1:10000, SigmaAldrich, T6074, clone B-5-1-2). Secondary antibodies: goat F(ab')2 anti-rabbit IgG (H+L) HRPO (1:10000, 111-036-045, Dianova), goat F(ab')2 anti-mouse IgG (H+L)-HRPO (1:10000, 115-036-062, Dianova).

Validation

Only commercially available antibodies were used that had been confirmed to be specific by the manufacturer and were tested again by us in zebrafish or rabbit reticulocyte samples for their specificity by Western Blotting (see Supplementary Figure 2).

Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research.

Laboratory animals

TLAB zebrafish, generated by crossing zebrafish AB with the natural variant TL (Tupfel Longfin), served as wild-type zebrafish for all experiments. Adult fish ranged in the age from 3 months to 1.5 years. Wild-type Xenopus laevis were obtained from NASCO (USA); a wild-type female of 5 years and 7 months was used in this study.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All fish and Xenopus experiments were conducted according to Austrian and European guidelines for animal research and approved by local Austrian authorities (animal protocols for work with zebrafish: GZ 342445/2016/1.2 and MA 58-221180-2021-16; animal protocols for work with Xenopus: BMWFW-66.006/0012-WFI/3b/2014, BMWFW-66.006/0003-WF/V/3b/2016).

Note that full information on the approval of the study protocol must also be provided in the manuscript.