Distinct Maternal and Somatic rRNA Types in Zebrafish Development.
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Summary

Chapter 1: Introduction
The aim of this thesis is defined as the discovery and analysis of variant 5S and 45S ribosomal RNA (rRNA) types in Zebrafish development. Therefore, the first part of this chapter is dedicated to an extensive review of the current knowledge on the biogenesis of the ribosome, its components and its functions. Also, the concept that rRNAs can have sequence heterogeneity is introduced. The reasons of the choice for the model organism to be analyzed, zebrafish, and its embryogenesis are briefly discussed. Next-generation sequencing (NGS) was chosen as the main technical approach, but standard laboratory protocols needed to be adjusted for the analysis of rRNA, and an explanation is presented here. Finally, an outline of the thesis and its results are given.

Chapter 2: Improving small RNA-seq by using a synthetic spike-in set for size-range quality control together with a set for data normalization
In our experience with NGS of small RNA (sRNA), sRNA-seq, we encountered two main challenges: how to check the size distribution of isolated sRNAs, given the sensitive size-selection steps in the library preparation protocol, and how to normalize data between samples, given the low complexity of sRNA types. To address these challenges, we here propose two separate sets of synthetic RNA spike-ins for monitoring size-selection and for performing data normalization in sRNA-seq. The size-range quality control (SRQC) spike-in set, consisting of 11 oligoribonucleotides (10–70 nucleotides), was tested by intentionally altering the size-selection protocol and verified via several comparative experiments. We demonstrate that the SRQC set is useful to reproducibly track down biases that can occur during the size-selection steps of sRNA-seq. The external reference for data-normalization (ERDN) spike-in set, consisting instead of 19 oligoribonucleotides (25 nucleotides), was developed for sample-to-sample normalization in differential-expression analysis of sRNA-seq data. Testing and applying the ERDN set showed that it can reproducibly detect differential expression. We used the knowledge acquired during the development of these controls to establish a reproducible protocol for sRNA-seq.
Chapter 3: Linking maternal and somatic 5S rRNA types with different sequence-specific non-LTR retrotransposons

It is known that some eukaryotic species have two 5S rRNA types defined by their predominant expression in oogenesis or adult tissue. Our sRNA-seq study on zebrafish egg, embryo and adult tissue, identified a maternal-type 5S rRNA that is exclusively accumulated during oogenesis, replaced throughout the embryogenesis by a somatic-type, and thus virtually absent in adult somatic tissue. The maternal-type 5S rDNA contains several thousands of gene copies on chromosome 4 in tandem repeats with small intergenic spacers, whereas the somatic-type is present in only 12 gene copies on chromosome 18 with large intergenic spacers. We propose that the nine-nucleotide variation between the two 5S rRNA types likely affects TFIII binding and riboprotein L5 binding, probably leading to storage of maternal-type rRNA, as shown before in Xenopus. Remarkably, the majority of these sequence differences are located at the sequence-specific target-site for genome integration by the 5S rRNA-specific Mutsu retrotransposon family. Thus, we could define maternal- and somatic-type MutsuDr subfamilies. Furthermore, we identified four additional maternal-type and two new somatic-type MutsuDr subfamilies, each with their own target sequence. This target-site specificity, frequently intact maternal-type retrotransposon elements, plus specific presence of Mutsu retrotransposon RNA and piRNA in egg and adult tissue, suggest an involvement of retrotransposons in achieving the differential copy number of the two types of 5S rDNA loci.

Chapter 4: Expression of Distinct Maternal and Somatic 5.8S, 18S and 28S rRNA Types during Zebrafish Development

We report here the discovery of maternal- and somatic-types for the 45S rDNA elements: 5.8S, 18S, and 28S, similar to what we previously found for 5S. The maternal-type 5.8S, 18S and 28S rRNA sequences differ substantially from those of the somatic-type, plus the maternal-type rRNAs are also replaced by the somatic-type rRNAs during embryogenesis. We discuss the structural and functional implications of the observed sequence differences with respect to the translational functions of the 5.8S, 18S, and 28S rRNA elements. Finally, in silico evidence suggests that two expansion segments (ES) in 18S rRNA, previously implicated in the ribosome – mRNA interaction, for each rRNA type may have a preference for interacting with specific mRNA genes. Taken together, these findings indicate that two distinct types of ribosomes exist in zebrafish during development, each likely conducting the translation machinery in a unique way.
Chapter 5: Identifying small RNAs derived from maternal- and somatic-type rRNAs in Zebrafish Development

In this chapter, we used sRNA-seq data obtained from various zebrafish developmental stages, to systematically investigate small RNAs originating from rRNAs (srRNAs) of maternal- or somatic-type 18S, 5.8S and 28S. We identified new srRNAs for each rRNA. For 5.8S, we found srRNA consisting of the 5’ or 3’ halves, with only the latter having a different sequence for the maternal- and somatic-types. For 18S, we discovered a 21 nt srRNA from the 5’ end of 18S rRNA that shows a striking resemblance to microRNAs as it is likely processed from a stem-loop precursor and also present in human and mouse Argonaute-complexed small RNA. For 28S, an abundant 80 nt srRNA from the 3’ end of the 28S rRNA was found. The expression levels during embryogenesis of these srRNAs indicate that they are likely not generated from rRNA degradation and thus might have a functional role in the zebrafish development.

Chapter 6: Conclusion

Finally, our findings are critically discussed. We present hypotheses on the transcriptional regulation of maternal- and somatic-type rRNAs during zebrafish development and on how their sequence differences might influence ribosome biogenesis, structure and function at different levels. Moreover, we focus on some preliminary results from our studies, like the presence of an unexpressed rRNA type in the zebrafish genome and the comparison with other vertebrate species. At the end of the chapter, developmentally-regulated rRNA types are put in the context of the “specialized ribosomes” theory.