

Transcriptional Regulation of Ribosomal RNA by a Germ Granule Component, *Meioc*, for Differentiation of Germline Stem Cells in Zebrafish

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Recent studies in both embryonic and adult stem cells demonstrated that stem cells require low translation rates to maintain the undifferentiated status. However, mechanisms used by stem cells to control translation are still unclear. Here we describe a novel ENU-generated mutant zebrafish in which germline stem cells (GSCs) fail to differentiate, and novel properties of the responsible gene *meioc* as a germ granule component that controls upregulation of rRNA transcripts.

Upregulation of 5.8S, 18S, 28S rRNAs was observed in 1-4-cell cyst spermatogonia in wild-type zebrafish, while the mutant maintained spermatogonia with low levels of those rRNAs. The encoded *Meioc* protein is observed in cytoplasmic granules from a portion of 1-2-cell cyst germ cells, and interacts with Piwil1 (*Ziwi*). Depletion of *Meioc* resulted in accumulation of Piwil1 in nucleoli. The nucleolar localization of Piwil1 was diminished by inhibition of RNA polymerase I activity, and Piwil1 associated with unprocessed pre-rRNA. Although the *piwil1*^{-/-} mutants have a germ cell-depleted testis, we found that heterozygous *piwil1*^{+/-} spermatogonia exhibited reduction of Piwil1. The *piwil1*^{+/-} spermatogonia upregulated rRNA transcripts, and the *piwil1*^{+/-};*meioc*^{-/-} spermatogonia then recovered differentiation later than those in *meioc*^{-/-}. Furthermore, Piwil1 interacted with HP1 α in nucleoli, and *meioc*^{-/-} spermatogonia exhibited high levels of H3K9me3 and methylated CpG in the 45S-rDNA region. These results indicate that zebrafish GSCs maintain low rRNA transcriptional activity with high H3K9me3 and methylated CpG, and that *Meioc* has a function of upregulating rRNA transcripts and promoting differentiation by preventing localization of Piwil1 and HP1 α in nucleoli.

All animal experiments were approved by the Animal Care and Ethics Committees of the National Institute of Genetics and carried out according to the guidelines.