# Chapter 15
# What are sperm-borne RNAs and do they play a role in germ cell function?

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Due to a lack of typical cytoplasm, spermatozoa have been thought to contain no ribonucleic acids (RNAs). However, over the past fifteen years, numerous studies have demonstrated that sperm indeed contain RNAs, which can be delivered into the oocytes during fertilization. Given that RNAs can be obtained from either the whole sperm or sperm heads with or without the plasm membrane, the sperm-borne RNAs must exist in both the sperm nucleus and non-nuclear compartments. The sperm nuclear RNAs are likely loaded during late spermiogenesis when transcription is shut down and the spermatid nucleus starts condensation and elongation, whereas the RNAs located in the non-nuclear compartments of the sperm, e.g., perinuclear theca, neck and flagellum, may derive from either the former cytoplasm, which gets shed upon spermiation (i.e., sperm release from the seminiferous epithelium), or from the cytoplasmic droplets, representing a highly specialized organelle as an integral part of the maturing epididymal sperm. Recent data suggest that sperm gain small RNAs from the exosomes of the epididymal epithelial cells when sperm transit through the epididymis for maturation, but the claim was primarily based on the dynamic changes in proportional distribution patterns of small RNAs in testicular, caput, and cauda epididymal sperm. Given that epididymal sperm mostly contain cytoplasmic droplets, which also contain a large number of large and small RNAs, it is imperative to examine whether the changing small RNA compositions between sperm from the caput and cauda epididymides are due to small RNA shuffling between the cytoplasmic droplets and sperm, or between the epididymosomes and sperm. Nevertheless, the sperm RNA payloads appear to be selective because the species and relative abundance of various RNAs differ between the sperm nucleus and non-nuclear compartments. It remains unknown how specific RNAs are selected and loaded into the sperm nucleus and other compartments during spermiogenesis and epididymal maturation.

Sperm contain both large (>200nt) and small (<200nt) RNAs, but sperm RNAs are much less abundant compared to those in somatic cells (~0.1-0.3 pg per human sperm vs. 10-30 pg in a typical somatic cell) (Fig. 1). Among the sperm-borne large RNAs, messenger RNAs (mRNAs), large noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) have been detected. Although full-length mRNAs have been identified through the latest third-generation long-range sequencing, most mRNAs in sperm appear to be truncated mRNA fragments in both linear and circular forms. Many sperm-borne circRNAs contain open reading frames, implying their potential to be translated into proteins. Indeed, peptides corresponding to these sperm-borne circRNAs have been identified through proteomics analyses. However, it remains to be determined whether these peptides are the products of the circRNAs. Given that circRNAs are resistant to degradation, it has been proposed that these circRNAs may function to maintain continued protein production when mRNAs are undergoing massive decay toward the end of spermiogenesis and are largely unavailable after spermiation.

Sperm-borne small RNAs are relatively more abundant than large RNAs in sperm. Numerous small RNA species have been identified, including microRNAs (miRNAs), endogenous small interfering RNAs (endo-siRNAs), piwi-interacting RNAs (piRNAs), tRNA-derived small RNAs (tsRNAs), rRNA-derived small RNAs (rsRNAs), mitochondrial genome-encoded small RNAs (mitosRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and many other unannotated small RNA species (Fig. 1). The relative abundance of various small RNAs species differs between whole sperm and sperm heads. For example, tsRNAs are more enriched in the sperm head, whereas mitosRNAs are always more abundant in the whole sperm. The differential small RNA distribution patterns within a sperm further support the notion that the sperm-borne small RNAs are selectively loaded into the nucleus and other non-nuclear compartments. Of interest, the sperm-borne small RNA profiles are different from those in their precursor cell types (e.g., spermatids), but appear to be conserved among closely related mammalian species, further supporting the concept that these small RNAs are purposely packaged into sperm and may have conserved physiological roles.

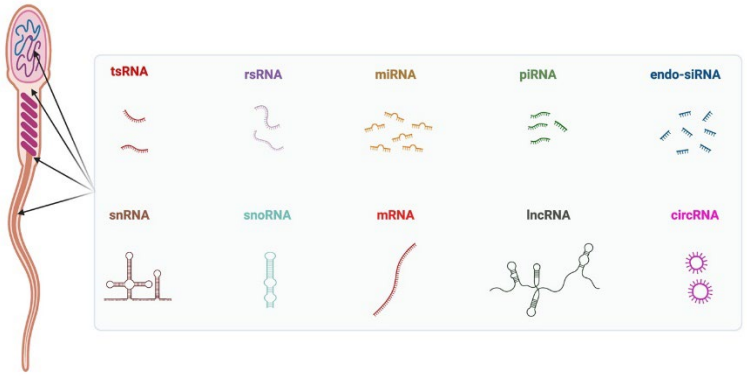


**Figure 1.** Types of RNAs and their locations in mammalian sperm.

Although sperm contain full-length mRNAs, it is highly unlikely that these sperm-borne mRNAs can be translated into proteins due to a lack of cytoplasm and the canonical, cytoplasmic translational machinery. A previous study reported that sperm may be able to translate their mRNAs into proteins using the mitochondrial translation machinery, but subsequent independent studies have failed to validate this claim. Alternatively, the sperm-borne mRNAs, in theory, can be translated into proteins upon delivery into the oocyte cytoplasm, thereby participating in fertilization and preimplantation embryonic development. However, this has not been demonstrated experimentally. Similarly, the potential functions of sperm-borne large noncoding RNAs remain to be investigated.

Sperm-borne miRNAs and endo-siRNAs have been shown to be important for fertilization and preimplantation embryonic development because partial depletion of miRNAs and endo-siRNAs in sperm causes compromised early embryonic development due to dysregulated transcriptomic profiles in fertilized eggs and early embryos. Since miRNAs and endo-siRNAs are known to function to regulate mRNA stability through complementary binding to the 3'UTRs of mRNAs, the dysregulated transcriptome of early embryos may result from sperm deficiency in miRNAs and endo-siRNAs. In addition to the canonical role of miRNAs and endo-siRNAs, sperm-borne small RNAs appear to mediate non-genetic inheritance of acquired paternal traits, including the disease phenotypes induced by environmental and dietary factors (e.g., glucose intolerance and

metabolic disorders induced by a high fat or a low protein diet,) and certain paternal behavior gained during the father's lifetime. More intriguingly, microinjection of mouse sperm-borne total RNAs, miRNAs, tsRNAs or rsRNAs into wild-type mouse oocytes appears to be able to recapitulate the paternal phenotypes in offspring, suggesting these sperm-borne small RNAs may function as the epigenetic information carrier responsible for the paternal transmission of the phenotypes acquired during a father's lifetime. However, it remains unclear how sperm-borne small RNAs act at molecular levels to induce the specific paternal phenotypes in offspring.

In summary, mammalian sperm contain RNAs, both large or small and coding or noncoding (Fig. 1). These RNAs are likely purposely loaded into sperm during spermatogenesis. Sperm-borne RNAs may have roles in supporting preimplantation embryonic development and as one of the epigenetic information carriers to mediate epigenetic inheritance of paternally acquired traits through environmental, dietary, and other factors.

## Suggested reading

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