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Transferring Fragments of Paternal Metabolism to the Offspring

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Paternal diet influences offspring metabolism, yet the underlying epigenetic mechanisms are unclear. Recently, Chen et al. (2016) and Sharma et al. (2016) identified tRNA fragments in sperm and the male reproductive tract as possible inherited factors altered by paternal diet that lead to gene misexpression and altered metabolism in offspring.

How the environment in which we live affects our health and, consequently, the health of our offspring is the focus of many recent lines of investigation. The mechanism is likely epigenetic, meaning that it occurs independent of changes to the DNA base sequence. Yet the inherited factor passed between generations via the germline is not well understood. Mammalian studies have largely focused on identifying DNA methylation patterns that change in response to environmental stressors and that appear in the offspring to cause misexpression of genes and a phenotype (Radford et al., 2014). Indeed, manipulating paternal diet can lead to altered metabolism in his progeny (Carone et al., 2010; Ng et al., 2010). However, attempts to correlate DNA methylation patterns in sperm to metabolic changes in the offspring have not been fruitful, suggesting other mechanisms are involved.

Now, two independent studies published in a recent issue of *Science* (Chen et al., 2016; Sharma et al., 2016) implicate cleaved transfer RNAs (tRNAs) in mouse sperm as an inherited epigenetic factor influenced by paternal diet and responsible for metabolic disruption in the offspring. Typically, full-length tRNAs are associated with ribosomes during the synthesis of new proteins. The cleaved versions (30–40 nucleotides in size) have been previously described in sperm (Peng et al., 2012), but their function in germ cells and later in offspring health was unclear. In both studies, RNA-sequencing of control sperm revealed an abundance of 5′ fragments of tRNA (tRFs), ranging from 64% to 80% of all small RNAs, including microRNAs and piwi-interacting RNAs (Chen et al., 2016; Sharma et al., 2016). Sharma et al. (2016) also revealed that tRFs gradually accumulate as sperm mature in epididymis of the male reproductive tract. Most importantly, the studies revealed an increase in sperm tRFs in response to paternal diet manipulation. While Chen et al. (2016) focused on a mouse model of high-fat diet leading to glucose intolerance and insulin resistance in offspring, Sharma et al. (2016) analyzed the outcome of a low-protein diet in mice, which was shown previously to alter the expression of offspring genes involved in hepatic lipid and cholesterol biosynthesis (Carone et al., 2010).

By microinjecting zygotes with tRFs isolated from the sperm of males fed a high-fat diet, Chen et al. (2016) revealed a direct link between sperm tRFs and offspring metabolic disorder (Figure 1). The metabolic phenotype was only partially recapitulated since these offspring exhibited impaired glucose tolerance but no insulin resistance. This suggests that paternal diet may affect glucose metabolism in the next generation via sperm tRFs, although another epigenetic mechanism is required to fully establish the phenotype. Regardless, RNA-sequencing of adult pancreatic islets from the offspring derived from tRF-microinjected zygotes uncovered downregulation of genes involved in ketone, carbohydrate, and monosaccharide metabolisms (Chen et al., 2016), emphasizing the potentially disruptive effect of sperm tRFs. To further understand how tRFs are regulated, Chen et al. (2016) identified RNA modifications (e.g., 5-methylcytodine and N^2-methylguanosine) that were more prevalent in tRFs isolated from high-fat diet sperm than controls. When synthesized without these modifications, these tRFs were unable to induce metabolic disorder, likely due to reduced RNA stability. As such, tRFs generated in sperm exposed to a high-fat diet might resist degradation and persist in the fertilized zygote (and beyond) to influence metabolism.

In an attempt to understand whether offspring metabolic disorder is rooted in

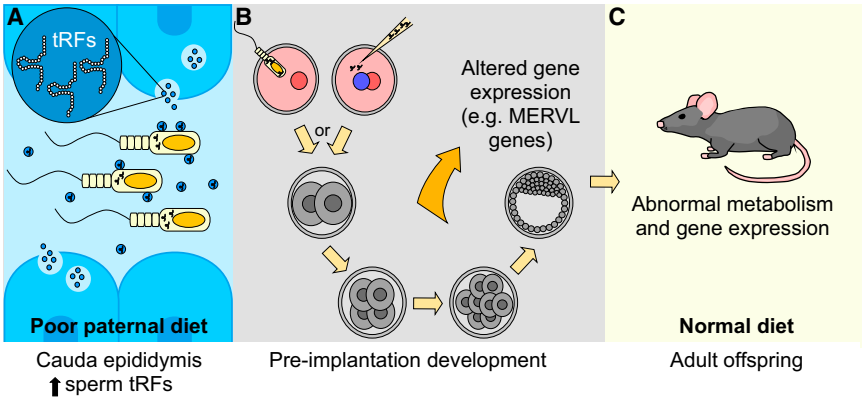

Figure 1. Poor Paternal Diet Increases tRNA Fragments in Sperm and the Male Reproductive Tract, Leading to Altered Gene Expression and Metabolic Disruption in Offspring

(A) Poor paternal diet, such as a high-fat or low-protein diet, increases the number of tRNA fragments (tRFs) in sperm compared to controls (Chen et al., 2016; Sharma et al., 2016). During sperm maturation, sperm fuse with exosomes secreted by the epididymis (epididymosomes), which also contain tRFs (Sharma et al., 2016).

(B) In vitro fertilization involving mature sperm from a male fed a poor diet or microinjection of tRFs isolated from similar sperm into control zygotes leads to altered gene expression in pre-implantation embryos (Chen et al., 2016; Sharma et al., 2016). Offspring genes that are particularly affected by sperm tRFs from males fed a low-protein diet are those regulated by the endogenous retroelement MERVL (Sharma et al., 2016).

(C) The offspring resulting from embryo manipulations observed in (B) exhibit abnormal metabolism and altered tissue-specific gene expression, even when fed a normal diet. In the case of offspring derived from tRFs of sperm from males fed a high-fat diet, adult pancreatic islet gene expression is altered and is associated with glucose intolerance (Chen et al., 2016).

sperm tRF-related dysregulation of gene expression during early offspring development, both Sharma et al. (2016) and Chen et al. (2016) microinjected sperm tRFs into zygotes to assess the transcriptional profiles of pre-implantation embryos (Figure 1). Chen et al. (2016) showed by RNA-sequencing that hundreds of genes were up- or downregulated in eight-cell and/or blastocyst-stage embryos derived from zygotes injected with sperm tRFs from males fed a high-fat diet. The downregulated genes were specifically enriched for metabolic regulatory pathways (Chen et al., 2016), the results of which correlate with another study showing that paternal obesity leads to impaired development of embryos at a similar stage (Mitchell et al., 2011). More definitively, Sharma et al. (2016) identified a specific fragment, tRF-Gly-GCC, that was upregulated in sperm from males fed a low-protein diet. Through a series of rigorous experiments, they showed that increased tRF-Gly-GCC in sperm was sufficient to disrupt expression of specific gene targets in two-cell embryos. These genes are regulated by the long terminal repeat of the endogenous retroelement MERVL (Sharma et al., 2016) and are known for their involvement in pluripotency (Macfarlan et al., 2012).

Intriguingly, Sharma et al. (2016) discovered that epididymosomes, exosomes released by epididymal cells, also contain abundant tRFs similar to those in mature sperm (including tRF-Gly-GCC). The tRF content of epididymosomes was unchanged even in spermless mice. Remarkably, the authors showed that by exposing spermatozoa isolated from the caput epididymis to purified epididymosomes isolated from the cauda epididymis, fusion occurred, resulting in the transfer of the epididymosomal tRF cargo into the sperm (Figure 1). Although it is unclear whether sperm have an intrinsic ability to cleave tRNA, the uptake of tRFs from epididymosomes illustrates a clear mode of communication between the paternal environment (i.e., nutrient availability) and the mature sperm. Sperm are transcriptionally silent due to their condensed nuclear structure and can persist in the male reproductive tract for ~70 days. Therefore, epididymal cell-sperm communication might convey an adaptive metabolic advantage to the offspring.

The importance of sperm small RNAs in mammalian epigenetic inheritance is becoming clear (Chen et al., 2016; Grandjean et al., 2015; Sharma et al., 2016). Further investigations are required to determine whether tRFs directly regulate gene expression and whether disruption of MERVL-regulated genes during pre-implantation development is sufficient to program the adult metabolism. Also, how this mechanism fits in with that of other inherited epigenetic factors (e.g., DNA and protamine methylation) to transmit information about the paternal environment requires additional investigation. Another exciting prospect will be to assess whether tRFs are inherited beyond the immediate offspring to influence metabolism in subsequent generations.

The occurrence of tRFs in human sperm is yet to be determined, though they are present and functional in other human cell types (Elbarbary et al., 2009). Nevertheless, conservation of tRFs in the male reproductive system across mammalian species is evident, since sperm and epididymosomal tRFs appear in both mouse and bull (Sharma et al., 2016). As obesity and diabetes rates rise in human populations, further evidence that paternal diet and offspring metabolism are linked is alarming. However, if the RNA mechanism described in these studies can be reversed, it will have major implications for health and disease prevention for many generations to come.

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