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A Twist between ROS and Sperm-Mediated Intergenerational Epigenetic Inheritance

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Abstract

Yoshida et al. (2020) report in this issue of *Molecular Cell* that a paternal low-protein diet elevates ROS in the testicular germ cells, altering ATF7 activity and H3K9me2 abundance on target genes, including tRNA loci. These changes are maintained in spermatozoa, regulating tsRNA biogenesis, and together transmit intergenerational effects.

Increasing evidence demonstrates that unhealthy paternal nutritional status, such as a high-fat, high-sugar, or low-protein diet in mammals can induce non-DNA sequence-based epigenetic changes in the sperm and cause metabolic disorders in the offspring (Zhang et al., 2019). However, a clear mechanism responsible for this intergenerational epigenetic inheritance phenomenon remains lacking. An interplay between histone marks, transcriptional activity, and small non-coding RNAs (sncRNAs) in the germ cell was anticipated to underlie such phenomena in mammals, as has been found in animals such as worms and flies. In this issue of *Molecular Cell*, Yoshida and colleagues report that a paternal low-protein diet (pLPD) elevates reactive oxygen species (ROS) in the mouse testicular germ cells (TGCs), altering transcription factor binding activity and associated histone marks that are maintained in spermatozoa, along with altered sperm sncRNA biogenesis, which may together transmit intergenerational effects.

The present work (Yoshida et al., 2020) extends from the lab's previous studies in a fly model in which they found that environmental stresses in *Drosophila* can induce phosphorylation of ATF-2 (a mammalian ATF7 homolog), resulting in decreased H3K9me2 levels and altered heterochromatic status that are transmitted to the offspring (Seong et al., 2011). To explore whether ATF7 plays a similar role in mammalian sperm-induced intergenerational epigenetic inheritance, the authors chose a pLPD mouse model that has been reported to induce intergenerational effects in F1 offspring, manifested by altered liver gene expression (Carone et al., 2010). They first repeated the paternal LPD versus control diet (CD) treatment and indeed observed altered liver gene expression in the F1 offspring from the LPD-treated sires (Figure 1A). Next, the authors applied this paternal LPD versus CD model in an *Atf7*$^{+/-}$ mutant background and tracked the liver gene expression pattern in the F1 offspring as a readout for the intergenerational effects, from which they showed two interesting lines of findings and raised new questions along the way.

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First, they found that the F1 *Atf7*$^{+/-}$ offspring from the *Atf7*$^{+/-}$ sires, regardless of sire's diet (LPD versus CD), showed a similar liver gene expression pattern largely overlapping with the F1 *Atf7*$^{+/+}$ offspring from the wild-type *Atf7*$^{+/+}$ LPD sires (Figure 1A). This phenomenon can be partially explained by their data that pLPD elevated the ROS in TGCs, which increased the phosphorylation of ATF7 by p38 and induced ATF7 release from its target; these changes in F0 *Atf7*$^{+/+}$ males may mimic the deletion (dosage) effect of *Atf7*$^{+/-}$. Supporting this idea, the authors found that the H3K9me2 abundance in TGCs was similarly decreased in the *Atf7*$^{+/+}$-LPD, *Atf7*$^{+/-}$-CD, and *Atf7*$^{+/-}$-LPD groups comparing to the *Atf7*$^{+/+}$-CD group. They further validated the altered ATF7 binding and H3K9me2 coverage in a set of metabolic-related genes in TGCs by ChIP-qPCR. Importantly, the change in H3K9me2 abundance on these genes was maintained in the mature sperm (spermatozoa). Notably, although ATF7 recruits both H3K9 di- and tri-methyltransferases, the overall H3K9me3 abundance in spermatozoa were not changed (Figure 1B).

These data suggest a scenario in which the pLPD-ROS-p38-ATF7 axis generates a signature of histone marks on the *Aft7*(+) sperm from pLPD-exposed wild-type *Atf7*$^{+/+}$ TGCs, similar to that of the *Atf7*(−) sperm from *Atf7*$^{+/-}$ TGCs (regardless of LPD or CD). This signature of histone marks might be responsible for programming embryo development, in that they induce the altered liver gene expression pattern in the F1 offspring. According to Yoshida et al., this intergenerational effect is most likely via an indirect mechanism through cascade regulation during development rather than by direct maintenance of H3K9me2 from sperm into the liver, due to the limited overlap of altered genes. The altered liver gene expression in F1 *Atf7*$^{+/-}$ offspring may also be due in part to the lack of one *Atf7* allele in the liver cells. Nonetheless, these data showed how an epigenetic mark generated during spermatogenesis upon environmental stress can be maintained in spermatozoa, which may stand as a general mechanism in addition to the recent idea that sperm can gain exosomal information during epididymal transition (Zhang et al., 2019).

The second finding is more puzzling. The authors found that the F1 *Atf7*$^{+/+}$ offspring from the F0 *Atf7*$^{+/-}$ sires under LPD versus CD showed substantially diminished changes in liver gene expression patterns compared to the distinct patterns between F1 *Atf7*$^{+/+}$ offspring from the F0 *Atf7*$^{+/+}$ sires under LPD versus CD (Figure 1A). That is, the differential gene expression signature tends to become assimilated toward an intermediate state. Since the *Atf7*(+) sperm derived from *Atf7*$^{+/+}$-LPD, *Atf7*$^{+/-}$-CD, and *Atf7*$^{+/-}$-LPD TGCs may have similar H3K9me2 status, the differential effects in F1 are difficult to explain by simply considering the perspective of histone marks. In particular, why did the deletion of one *Atf7* allele (*Atf7*$^{+/-}$) in addition to pLPD exposure attenuate the abnormal liver gene expression, instead of enhancing the effect?

One possible explanation is that beyond the effects of histone marks, *Atf7*$^{+/-}$ TGCs may generate additional (mobile) molecules that are inherited by *Atf7*(+) sperm (e.g., sncRNAs), which may act *in trans* to program embryo development. For example, the abnormal non-coding RNAs generated in the *Kit*$^{+/-}$ heterozygotic testis can be carried by the wild-type *Kit*(+) sperm to changethe offspring's phenotype as a case of paramutation in mice (Rassoulzadegan et al., 2006). Indeed, Yoshida et al. have found that the *Atf7*$^{+/-}$ TGCs showed altered transcriptional activities on a set of tRNAs compared to *Atf7*$^{+/+}$ TGCs,

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partially due to differential H3K9me2 coverage; and that the spermatozoa from *Atf7*$^{+/-}$ males showed altered levels of tRNA-derived small RNAs (tsRNAs), some of which respond differently to LPD versus CD (Figure 1B).

The altered tsRNAs in *Atf7*(+) sperm from *Atf7*$^{+/-}$ TGCs may exert an "assimilating" effect that, on the one hand, makes the *Atf7*(+) sperm from *Atf7*$^{+/-}$-CD males "less normal," while making the *Atf7*(+) sperm from *Atf7*$^{+/-}$-LPD males "less abnormal," thus resulting in a loss of gene expression distinction in the F1 offspring (Figure 1A). This scenario, while intriguing, is largely speculative because how the tsRNA may alter F1 gene expression via embryo development is thus far unclear, although injection of tsRNA-enriched sperm RNA fractions has been shown to induce metabolic phenotypes in the offspring (Chen et al., 2016). tsRNAs may regulate the establishment of histone marks and transcriptional and translational machinery in the early embryo, which are key questions awaiting to be addressed.

The authors also noticed that the change of some tRNAs in TGCs does not match that of their corresponding tsRNAs in spermatozoa. This could be a result of complicated regulation of sncRNA biogenesis in sperm by ROS: 1) ROS regulate the p38-ATF7-H3K9me2 axis to alter tRNA transcription. 2) ROS mediate the cleavage of tRNA into tsRNAs (Thompson and Parker, 2009), which depends on factors including tRNA modifications (Zhang et al., 2018), the specific RNases, and the compartmentalization of tRNAs (e.g., sperm head versus mitochondria) (Nätt et al., 2019). ROS also induce rRNA cleavage into rRNA-derived small RNAs (rsRNAs) (Thompson and Parker, 2009), and sperm rsRNAs are similarly altered by paternal diet and may function together with tsRNAs (Nätt et al., 2019; Zhang et al., 2018). 3) ROS impact broadly on the quality of sperm nucleus involving DNA methylation and DNA damage (Drevet and Aitken, 2020), which may confound the observation (Figure 1C). Additionally, RNA-sequencing bias when dealing with highly modified RNAs such as tRNAs/tsRNAs remains an unmet challenge when quantitatively interpreting the sncRNA-seq data (Zhang et al., 2019).

Finally, the involvement of ROS in epigenetic inheritance is thought provoking, because in addition to unhealthy diets, other environmental exposures including cigarette smoking and toxicants (e.g., arsenite and endocrine disruptors) may all convergently generate ROS in the developing germ cells, and ROS may function as a nexus for transmitting parental environmental stressors to offspring by integrating multiple epigenetic mechanisms. Admittedly, how exactly the sperm histone marks and sncRNAs interact to convey mammalian epigenetic inheritance remains elusive, but the conserved mechanism suggested in both flies and mice here (Seong et al., 2011; Yoshida et al., 2020) sheds a ray of light before the door finally opens for mammals, if open it will.

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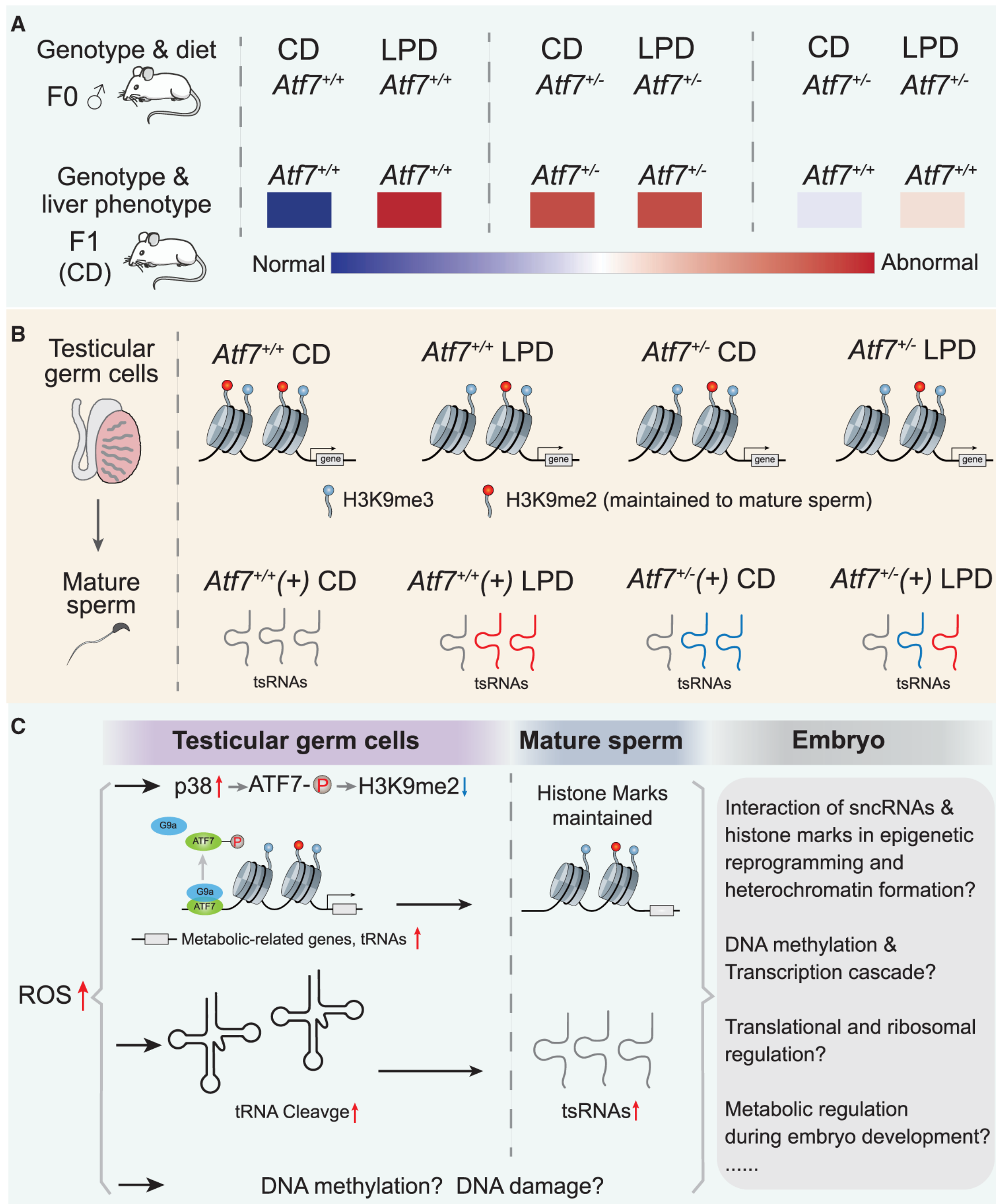

Figure.1. An Interplay between ROS, Histone Marks, and sncRNAs in Conducting Epigenetic Inheritance in Mice

(A) pLPD and the effect of ATF7 genotype in inducing intergenerational effects on liver expression pattern in F1 offspring.

(B) The mechanisms of intergenerational effects involve altered H3K9me2 in TGCs that are maintained in the spermatozoa and the altered tsRNAs in spermatozoa.

(C) Multifaceted roles of ROS in altering sperm epigenetic information carriers that may lead to environmental-stressor-induced mammalian epigenetic inheritance.