Stressed dads affect offspring brain development through sperm microRNA

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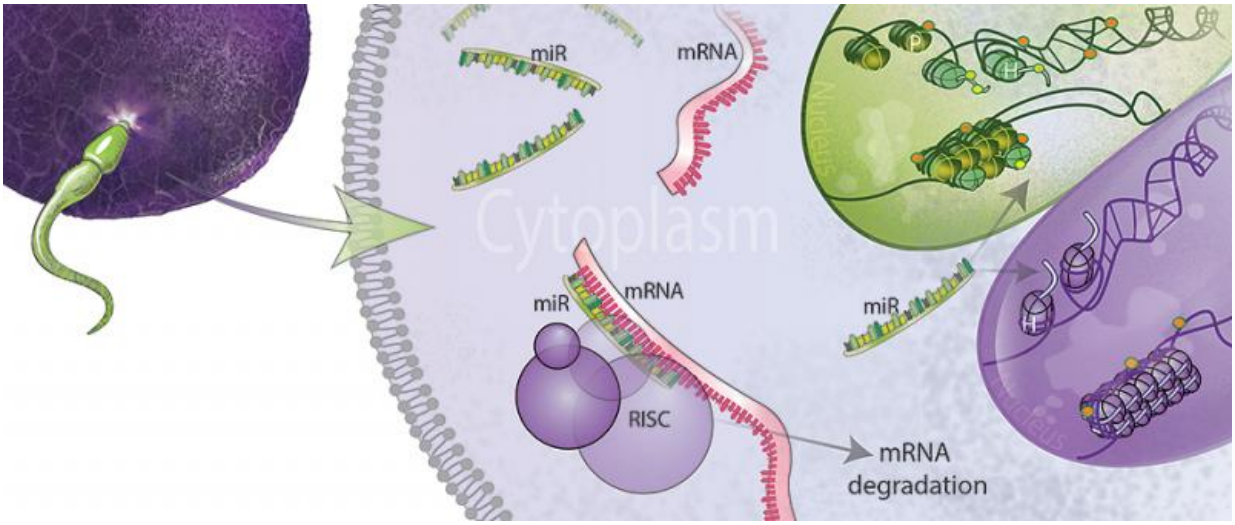

University of Pennsylvania researchers have shown at the molecular level how experiencing stress changes a male mouse's sperm in such a way that it affects his offspring's response to stress. This change is imparted epigenetically, or through a means other than the DNA code, by molecules called microRNAs, or miRs. Credit: University of Pennsylvania

More and more, scientists have realized that DNA is not the only way that a parent can pass on traits to their offspring. Events experienced by a parent over a lifetime can also have an impact.

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molecular level how experiencing stress changes a male mouse's sperm in such a way that it affects his offspring's response to stress. This change is imparted epigenetically, or through a means other than the DNA code, by molecules called microRNAs, or miRs.

The work, led by Tracy L. Bale, professor of neuroscience in Penn's School of Veterinary Medicine and Perelman School of Medicine, provides important clues for understanding how a father's life experiences may affect his children's brain development and mental health through a purely biological and not behavioral means.

"It's remarkable to me that seemingly mild stress to a male mouse would trigger this massive change in microRNA response and that that would get wired into the course of his offspring's development," Bale said.

She collaborated on the work with graduate students Ali B. Rogers and Christopher P. Morgan and research specialist N. Adrian Leu of Penn Vet. The paper will appear in *Proceedings of the National Academy of Sciences*.

In earlier research, Bale's lab had shown that male mice that were stressed, prior to being bred, by such means as changing cages or exposing them to a predator odor of fox urine, had offspring with a dampened response to stress. When they compared sperm from the stressed fathers to their unstressed counterparts, they found increased expression of nine miRs in the stressed animals. Unlike some other types of RNA, miRs do not code for a protein; instead, they serve to silence or degrade specific messenger RNAs, preventing them from being translated into proteins.

"Just showing that the levels were different doesn't make it relevant or interesting," Bale said. "We wanted to find out whether they were having a causal role."

To find out, the team microinjected the nine miRs into mouse zygotes, which were then implanted into normal female mice who carried them as surrogates. They also included control groups in which zygotes received either a sham injection or an injection of a single miR. When the offspring became adults, the researchers examined their response to stress, just as they had done in their 2013 study.

"The results mapped right onto what we had shown before," Bale said.

When subjected to a mild stress, in this case, being restrained briefly, the offspring that arose from the zygotes that received the multi-miR injections had lower cortisone levels compared to offspring in the control groups. The mice in the multi-miR injection group also had significant changes in the expression of hundreds of genes in the paraventricular nucleus, a brain region involved in directing stress regulation, suggesting wide-spread changes in early neurodevelopment.

Finally, the researchers aimed to determine how the miRs were carrying out this effect after fertilization. Because miRs are known to target and degrade mRNA, the team looked at the stored maternal mRNA, a genetic bundle that is contained in the egg when it is fused with the sperm and exists for only a brief window of time to direct early zygotic development.

"People used to think that because that stored maternal mRNA gets translated during that initial two-cell and four-cell development, the mom gets a lot of say in those early stages and the dad gets no say," Bale said. "But we thought maybe these sperm miRs could be attacking that maternal mRNA and directing which mRNAs get translated."

The researchers again injected miRs into zygotes and performed control injections, but this time they incubated the zygotes for eight hours and then amplified the RNA in each single cell to look for gene expression

levels. They found that, indeed, the multi-miR injection appeared to be attacking the maternal mRNA, resulting in a reduction in those mRNA levels compared to control injections. Specifically affected were genes involved in chromatin remodeling.

Bale suspects that when a male experiences stress it may trigger the release of miRs contained in exosomes from the epithelial cells that line the epididymis, the storage and maturation site for sperm between the testes and the vas deferens. These miRs may be incorporated into the maturing sperm and influence development at fertilization.

Up next for the group, including Penn Vet graduate student Jen Chan, who is taking over the project, is to examine what upstream factors could lead to exosome and miR release and whether an intervention, such as providing stressed males with enrichment or a reward, might prevent them from passing on an abnormal stress response to the next generation.

They also hope to study the role of miRs in humans to discern whether some may vary in response to stress in a similar way as in mice.

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More information: Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1508347112

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