within an experimental group. Measurement variability is often considered "noise" stemming from technical sources, but variability may also occur when perturbations cause disruptions without consistent directional change (hyper- or hypomethylation) in different individuals. Standard statistical methods may not be aimed at identifying unusually variable regions, or these may not be interpreted as potential contributors to regulatory dysfunction. Examining the presence and distribution of variability in epigenetic regulatory marks may enable us to identify the parts of the genome that are most susceptible to perturbation by environmental exposures.

In summary, Martínez et al. provide convincing evidence that maternal health has significant repercussions not only for the metabolic health of offspring, but also for the subsequent generation, as epigenetic regulatory modifications may be transmitted through gametes. The work prompts us to critically examine the evidence supporting widely accepted fundamental concepts in biology and to make use of an ever-expanding armamentarium of investigative tools available to us. Finally, the demonstration of the multigenerational effects of poor maternal nutrition gives us to reason to reflect on how mother-child health is prioritized from a public health policy perspective.

REFERENCES

Barker, D.J., and Thornburg, K.L. (2013). Clin. Obstet. Gynecol. *56*, 511–519.

Baron, U., Türbachova, I., Hellwag, A., Eckhardt, F., Berlin, K., Hoffmuller, U., Gardina, P., and Olek, S. (2006). Epigenetics *1*, 55–60.

Chédin, F. (2011). Prog. Mol. Biol. Transl. Sci. *101*, 255–285.

Houseman, E.A., Accomando, W.P., Koestler, D.C., Christensen, B.C., Marsit, C.J., Nelson, H.H., Wiencke, J.K., and Kelsey, K.T. (2012). BMC Bioinformatics *13*, 86.

Houseman, E.A., Molitor, J., and Marsit, C.J. (2014). Bioinformatics *30*, 1431–1439.

Martínez, D., Pentinat, T., Ribó, S., Daviaud, C., Bloks, V.W., Cebrià, J., Villalmanzo, N., Kalko, S.G., Ramón-Krauel, M., Díaz, R., et al. (2014). Cell Metab. *19*, this issue, 941–951.

Wang, L., Zhang, J., Duan, J., Gao, X., Zhu, W., Lu, X., Yang, L., Zhang, J., Li, G., Ci, W., et al. (2014). Cell *157*, 979–991.

Father-Son Chats: Inheriting Stress through Sperm RNA

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Although mounting evidence in mammals suggests that certain ancestral environmental exposures can influence the phenotype in future generations, mechanisms underlying such intergenerational information transfer remain unclear. A recent report suggests that RNA isolated from sperm can inform offspring of a father's history of early life trauma (Gapp et al., 2014).

The last decade has seen a dramatic resurgence of the once-discredited idea that ancestral environmental conditions can influence the phenotype of future generations—such intergenerational transfer of information is often called "the inheritance of acquired characters," or (incorrectly) "Lamarckian inheritance." As mothers play a far greater role in provisioning for early development than fathers, particularly in mammals, maternal environmental conditions can impact offspring both via environmentally directed molecular changes in the oocyte and by direct effects of maternal factors on the developing fetus. In contrast, fathers contribute mostly just sperm to the developing offspring, making the mechanisms responsible for intergenerational information transfer in paternal effect paradigms both experimentally tractable and of great interest (Rando, 2012).

An expanding number of paradigms link paternal environmental exposures to phenotypic traits in offspring, with the two dominant types of exposure history being dietary perturbations (high-fat diet, etc.) and various psychological stress conditions. Examples of the latter include chronic variable stress and social-defeat stress (Dietz et al., 2011; Rodgers et al., 2013). In a recent issue of *Nature Neuroscience*, Mansuy and colleagues focus on paternal effects using an "MSUS" paradigm—maternal separation coupled with unpredictable maternal stress—to induce early life stress (Gapp et al., 2014). Male mice subject to MSUS showed depression-like behavior in adult life—spending more time floating in a forced swim test, for example—and passed on a depressive proclivity to their progeny. In addition, metabolic disorders are a common outcome of early life stress, and both MSUS males and their offspring exhibited reduced insulin levels at baseline.

How do paternal diet and stress influence offspring? The likeliest scenario is that epigenetic information is delivered to the zygote by sperm, although alternative information carriers such as seminal fluid are often overlooked in such studies.

Indeed, in very few cases has the role of sperm in paternal effect paradigms been directly tested experimentally via in vitro fertilization or related methods (Dias and Ressler, 2014; Dietz et al., 2011). That said, focusing on the likeliest hypothesis that the sperm epigenome is responsible, work from model organisms has identified multiple epigenetic information carriers—cytosine methylation, transcription factors, chromatin structure, RNA molecules, and prions—whose status in mammalian sperm could respond to environmental conditions. Multiple studies have reported changes in sperm cytosine methylation or RNAs in response to environmental conditions, but thus far no studies have successfully carried out *functional* tests of such epigenomic changes. Testing the sufficiency or necessity of an epigenetic change in sperm is extremely challenging for cytosine methylation, but for small RNAs such a test can be relatively conveniently carried out by injecting RNAs directly into zygotes.

This is the key contribution of Gapp et al. (2014), as they isolated total RNA from control and MSUS sperm and injected these populations into control zygotes. A subset of the phenotypes passed on via natural matings—increased time spent floating in the forced swim test, decreased weight, and altered serum glucose levels after stress—were recapitulated under these conditions. This observation is intriguing given the extensive evidence in model organisms implicating RNAs as the heritable molecule in various transgenerational epigenetic inheritance systems, and microinjection studies in mammals implicating noncoding RNAs in multigenerational impacts of genetic defects (Heard and Martienssen, 2014; Rando, 2012). Supported by these prior studies, the Gapp et al. data provide the first concrete evidence (to our knowledge) for RNA molecules being responsible for paternal passage of environmental information in mammals. To address the identity of the RNAs responsible for this effect, Gapp et al. analyzed small non-coding RNAs (sncRNAs) in sperm as the potential information carriers of stress to the subsequent generations. Comparisons between control and MSUS sperm revealed modest changes in tens of microRNAs. Several of these microRNAs were also misregulated in various tissues isolated from the offspring of MSUS males, although the meaning of this is unclear.

While total RNA injections recapitulate some of the behavioral and metabolic consequences of MSUS on offspring, it seems unlikely that the microRNA changes described in sperm are responsible for the offspring phenotypes induced. First, microRNAs are a minor component of sperm small RNAs (García-López et al., 2014; Peng et al., 2012). Along with the dramatic difference in cytoplasmic volume between sperm and oocyte, the relatively small amount of RNA in sperm, and high concentrations of microRNAs necessary for activity in vivo (Wee et al., 2012), this makes 2-fold changes in nonabundant microRNAs unlikely to have much regulatory effect on the phenotype of the zygote (Amanai et al., 2006). Second, mammals do not carry RNA-dependent RNA polymerase, responsible for RNA signal persistence over cell divisions in plant and worm systems. This raises the question of how microRNAs that change in sperm might eventually alter translation of specific mRNAs as reported in the hippocampus of MSUS offspring. Finally, the authors report that behavioral traits persist to grandchildren of MSUS males, yet microRNA changes are not seen in the sperm of sons. In other words, paternal exposure to MSUS induces a behavioral alteration in both sons and grandsons, yet the RNAs that change in the father's sperm are unchanged in the sperm of sons. This means either that these RNAs are not responsible for the transfer of information from father to son or (less likely) that perhaps the fathers send information to sons via RNAs, but sons pass similar behavioral information to grandsons by an alternative mechanism. Thus, the specific molecule, or the mix of molecules (paternal effect traits might result from many contributing molecules of small individual impact, similar to complex genetic traits), remains to be identified from the total RNA pools from MSUS sperm that can induce behavioral and metabolic phenotypes in offspring. Injections of specific sncRNAs into zygotes will be the next logical step.

Whatever the identity of the key RNAs, the strength of the Gapp et al. (2014) study is the demonstration that sperm RNAs have the potential to transmit some behavioral traits to the progeny, which raises many interesting questions. How does stress alter the RNA profile of MSUS sperm—how does the central nervous system communicate with the testis to alter spermatogenesis? Is RNA production or stability modulated during spermatogenesis in response to hormonal signaling, or could RNA molecules generated elsewhere somehow cross the blood-testis barrier to be delivered to sperm (Dias and Ressler, 2014)? Finally, how do changes in sperm RNA populations impact the zygote to eventually give rise to behavioral changes in the offspring?

Gapp et al. (2014) provide strong evidence for RNA as a mediator of paternal effect traits, and pave way for future studies to decipher the specific mechanism linking sperm RNAs to offspring phenotypes.

REFERENCES

Amanai, M., Brahmajosyula, M., and Perry, A.C. (2006). Biol. Reprod. *75*, 877–884.

Dias, B.G., and Ressler, K.J. (2014). Nat. Neurosci. *17*, 89–96.

Dietz, D.M., Laplant, Q., Watts, E.L., Hodes, G.E., Russo, S.J., Feng, J., Oosting, R.S., Vialou, V., and Nestler, E.J. (2011). Biol. Psychiatry *70*, 408–414.

Gapp, K., Jawaid, A., Sarkies, P., Bohacek, J., Pelczar, P., Prados, J., Farinelli, L., Miska, E., and Mansuy, I.M. (2014). Nat. Neurosci. *17*, 667–669.

García-López, J., de Dios Hourcade, J., Alonso, L., Cárdenas, D.B., and Del Mazo, J. (2014). Biochim. Biophys. Acta. Published online April 24, 2014. http://dx.doi.org/10.1016/j.bbagrm.2014.04.006.

Heard, E., and Martienssen, R.A. (2014). Cell *157*, 95–109.

Peng, H., Shi, J., Zhang, Y., Zhang, H., Liao, S., Li, W., Lei, L., Han, C., Ning, L., Cao, Y., et al. (2012). Cell Res. *22*, 1609–1612.

Rando, O.J. (2012). Cell *151*, 702–708.

Rodgers, A.B., Morgan, C.P., Bronson, S.L., Revello, S., and Bale, T.L. (2013). J. Neurosci. *33*, 9003–9012.

Wee, L.M., Flores-Jasso, C.F., Salomon, W.E., and Zamore, P.D. (2012). Cell *151*, 1055–1067.