Science
AAAS

You are what you eat, but what about your DNA?

Martha Susiarjo and Marisa S. Bartolomei

Science **345**, 733 (2014);
DOI: 10.1126/science.1258654

they can begin to assemble to form tertiary structures. In the case of designed RNAs, once modular building blocks (i.e., tiles) fold, they can join with other units to form larger repeating structures using stereochemically precise long-range interactions programmed into the sequence.

Geary *et al.* have artfully used these principles of RNA architecture, modularity, and folding to design planar, extensible RNA tiles that can be synthesized as continuous strands and can fold cotranscriptionally to form modular units. The tiles themselves are programmed to fold through the formation of modules. The modules are carefully positioned in the secondary structure to mediate internal tertiary interactions (see the figure) taking the place of the staple strands used in DNA origami. Self-assembly of tiles into supramolecular structures is achieved by positioning sequences on the periphery of individual tiles that form addressable and programmable "kissing hairpin" interactions, the RNA equivalent of DNA "sticky ends."

The work of Geary *et al.* is particularly timely because it provides a valuable new tool for the rapidly growing field of synthetic biology, which seeks to develop and apply new engineering principles to modify and improve existing forms of life. Up to now, developments in synthetic biology have been mostly limited to sequence-based tinkering with gene expression programs, with only rare forays into explicit use of 3D architectural modules (*11*, *12*). Many new types of RNAs have been discovered in just the last few years, but understanding of their roles and mechanisms of action has lagged behind because of lack of adequate tools to manipulate RNA in vivo. The approach of Geary *et al.* should allow nanotechnologists and synthetic biologists to apply much of what has been learned working with DNA to RNA to create tools to move this technology into living cells and organisms. These tools should revolutionize our understanding of the cell, and perhaps of life itself. ■

ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

REFERENCES

1. C. Geary, P. W. K. Rothemund, E. S. Andersen, *Science* **345**, 799 (2014).
2. N. C. Seeman, N. R. Kallenbach, *Biophys. J.* **44**, 201 (1983).
3. E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **394**, 539 (1998).
4. J. Zheng *et al.*, *Nature* **461**, 74 (2009).
5. P. W. Rothemund, *Nature* **440**, 297 (2006).
6. B. Wei, M. Dai, P. Yin, *Nature* **485**, 623 (2012).
7. M. Endo *et al.*, *Angew. Chem. Int. Ed. Engl.* (2014)
8. T. Yamazaki, J. G. Heddle, A. Kuzuya, M. Komiyama, *Nanoscale* **6**, 9122 (2014).
9. N. B. Leontis, E. Westhof, *RNA* **7**, 499 (2001).
10. A. Chworos *et al.*, *Science* **306**, 2068 (2004).
11. T. Hara, H. Saito, T. Inoue, *Chem. Commun. (Camb.)* **49**, 3833 (2013).
12. Y. Krishnan, F. C. Simmel, *Angew. Chem. Int. Ed. Engl.* **50**, 3124 (2011).

10.1126/science.1257989

EPIGENETICS

You are what you eat, but what about your DNA?

Parental nutrition influences the health of subsequent generations through epigenetic changes in germ cells

By **Martha Susiarjo** *and* **Marisa S. Bartolomei**

Human and animal studies have demonstrated that the prenatal environment affects adult health and disease. Epidemiological studies have shown that gestational exposure to maternal starvation or overnutrition of the paternal grandfather is linked to increased risks for cardiovascular diseases and diabetes (*1*, *2*). In both cases, adverse metabolic health outcomes can be transmitted multigenerationally. As well, pregnant rats fed low-protein diets produced two sequential generations of offspring that became diabetic as adults (*3*). Nevertheless, despite considerable research efforts elaborating the phenotypic consequences of in utero insults to adult offspring and to their progeny, the mechanisms mediating multigenerational effects are unclear. On page 785 of this issue, Radford *et al.* (*4*) undertook an in-depth, genome-wide approach using a mouse model of undernutrition. This model has been linked to low birth weight, glucose intolerance, and reduced pancreatic function in two subsequent generations (*5*). Radford *et al.* not only provide convincing mechanistic insights about the transmission of phenotypes to later generations, their findings also suggest a path forward for pursuing these types of detailed studies.

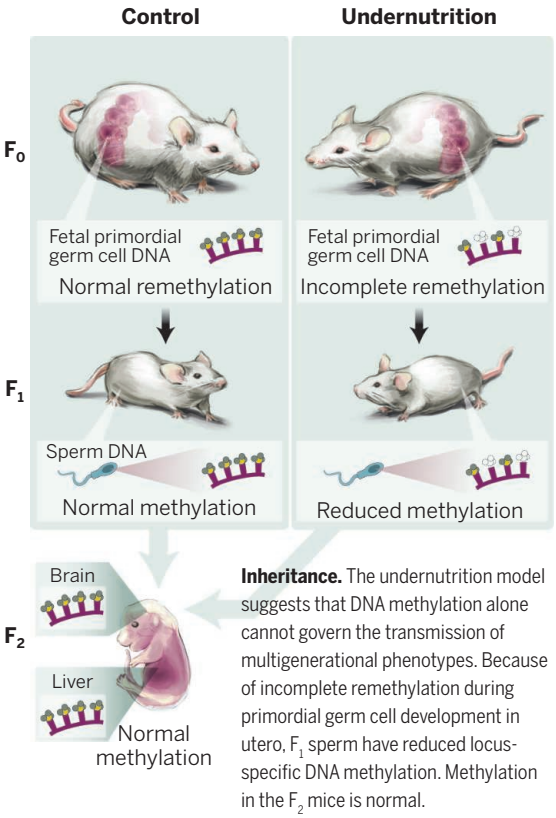
Because prenatal exposures are associated with adverse phenotypes much later in life, it is postulated that epigenetic mechanisms are involved. That is, heritable changes in DNA that are not accompanied by a change in DNA sequence could be responsible for remembering the insult. Epigenetic mechanisms include DNA methylation and changes in chromatin structure, noncoding RNA, and nuclear organization. The epigenetic mechanism commonly implicated in heritable transmission of a phenotype is DNA methylation. Other fetal exposure models have been associated with altered DNA methylation at the *IGF2* gene in humans (*1*) and the *PPARα* gene in rats (*6*). As such, in the absence of further environmental insults, one potential mechanism of how fetal perturbation influences the health of the subsequent generations is through germline epigenetic inheritance. Thus, the epigenetic signature of the sperm or oocyte from individuals who were reprogrammed in utero (i.e., the F_1) is transmitted to the next generation (i.e., the F_2).

To test the hypothesis that altered DNA methylation mediates the phenotypes of the undernutrition mouse model, Radford *et al.* used methylated DNA immunoprecipitation (MeDIP)–sequencing and bisulfite pyrosequencing and show that in utero caloric restriction in parent mice affects locus-specific DNA methylation patterns in the sperm from their offspring (adult F_1 mice) (see the figure). The nutritional stress occurred during late

gestation when primordial germ cells are epigenetically reprogrammed and are reacquiring DNA methylation specifically in the male germ line. Two independent pools of sperm derived from four representative litters per pool (one mouse per litter) were compared between control and undernutrition groups. A total of 111 hypomethylated regions from the nutritionally restricted F_1 males were identified. Of 24 randomly selected hypomethylated regions, 17 were validated, indicating that ~70% of hypomethylated candidate sequences were true differentially methylated regions. Although hypermethylated regions were also identified, none were validated when assayed by bisulfite pyrosequencing, demonstrating the importance of validation of candidate sequences by an alternative method on independent samples.

The presence of novel hypomethylated regions suggested that primordial germ cells from nutritionally restricted fetuses did not completely remethylate their DNA. These hypomethylated regions were sequences that remethylate later in normal primordial germ cells (*7*); hence, adverse fetal environment

Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. E-mail: bartolom@mail.med.upenn.edu



Inheritance. The undernutrition model suggests that DNA methylation alone cannot govern the transmission of multigenerational phenotypes. Because of incomplete remethylation during primordial germ cell development in utero, F_1 sperm have reduced locus-specific DNA methylation. Methylation in the F_2 mice is normal.

perturbed the epigenome nonrandomly. Furthermore, 21% of the hypomethylated regions overlapped with regions previously shown to be nucleosome-enriched (*8*), which is striking, because 99% of histones are normally replaced by protamines in mature sperm to facilitate packaging. The observation suggested that nutritional restriction in utero may have altered chromatin architecture of the sperm.

To determine whether the altered F_1 sperm epigenetic state could be transmitted to the F_2 generation, Radford *et al.* mated young, prediabetic F_1 males with control females and then assessed DNA methylation in F_2 liver and brain at embryonic day 16.5 (E16.5). The use of a paternal transmission strategy excluded maternal effects during pregnancy. Analysis of late embryonic F_2 tissues demonstrated that DNA methylation at the differentially methylated regions was reset and reprogrammed such that, by E16.5, methylation between control and food-restricted F_2 offspring was similar. However, a few genes in close proximity to the differentially methylated regions still displayed differential expression at E16.5. Together, the data suggest that DNA methylation may not be the primary epigenetic mechanism underlying the inherited gene expression profile and phenotypes in the F_2 offspring, although this remains to be determined. These observations are in contrast to results from a study that used a similar undernutrition mouse model in which a slight differential methylation at the *Lxra* locus was maintained in the F_2 generation (*9*). That study, however, used a candidate approach by first identifying transcriptional differences between control and undernutrition groups and then assaying candidate sequences. Differences in mouse chow ingredients and husbandry conditions could also contribute to the discrepancies between studies.

How nutritional deficiency in utero leads to multigenerational phenotype transmission remains unclear. The presence of hypomethylated regions in the F_1 sperm suggests that DNA methylation initially mediates environmental perturbation–induced developmental changes, but secondary epigenetic mechanisms must be involved. DNA methylation changes at other regions not detected by MeDIP sequencing may also be relevant to the affected developmental loci. Alternatively, other epigenetic modifications operate at these loci and mediate the inheritance of phenotypes. Histone H3 Lys^4 and Lys^{27} trimethylation (H3K27me3 and H3K4me3, respectively) are found in nucleosome-enriched developmental loci in sperm (*8*), implicating histone modifications as a potential mechanism for paternal transmission to the next generation. Another possible mechanism could involve small RNAs, as shown in a *Caenorhabditis elegans* caloric restriction model (*10*).

Radford *et al.* provide a model of how whole-genome approaches followed by independent validation should be conducted in analogous studies. Although DNA methylation plays an important role in nutritional restriction–induced developmental changes, other epigenetic mechanisms mediating multigenerational inheritance should be investigated. ■

REFERENCES

1. T. J. Roseboom *et al.*, *Mol. Cell. Endocrinol.* **185**, 93 (2001).
2. G. Kaati, L. O. Bygren, S. Edvinsson, *Eur. J. Hum. Genet.* **10**, 682 (2002).
3. K. A. Lillycrop, E. S. Phillips, A. A. Jackson, M. A. Hanson, G. C. Burdge, *J. Nutr.* **135**, 1382 (2005).
4. E. J. Radford *et al.*, *Science* **345**, 1255903 (2014); 10.1126/science.1255903.
5. J. C. Jiménez-Chillaron *et al.*, *Diabetes* **58**, 460 (2009).
6. H.-J. Park *et al.*, *J. Clin. Invest.* **118**, 259 (2008).
7. S. Seisenberger *et al.*, *Mol. Cell* **48**, 849 (2012).
8. S. Erkek *et al.*, *Nat. Struct. Mol. Biol.* **20**, 868 (2013).
9. D. Martínez *et al.*, *Cell Metab.* **19**, 941 (2014).
10. O. Rechavi *et al.*, *Cell* **158**, 277 (2014).

10.1126/science.1258654

WATER TREATMENT

Replace contamination, not the pipes

Rethinking water treatment additives can have synergistic benefits for urban water management systems

By **Wolfgang Rauch** *and* **Manfred Kleidorfer**

Wastewater from urban settlements contains—among a multitude of other substances—sulfate (SO_4^{2-}). Under anaerobic conditions, SO_4^{2-} can be biologically converted into toxic hydrogen sulfide gas (H_2S) and further to corrosive sulfuric acid (H_2SO_4), which results not only in noxious odors but also health issues and damage to sewer systems. This "sulfide problem" in sewers has long been recognized, but until recently, efforts have focused only on mitigation strategies for sulfide emissions in sewers. On page 812 of this issue, Pikaar *et al.* (*1*) provide an alternative to current technical measures—source control. They argue that by using substitutes for SO_4^{2-}, which is often used as a coagulant in the treatment of water, the SO_4^{2-} concentration in the wastewater can be reduced such that H_2S no longer affects sewer infrastructure.

Traditional urban water management usually involves the following (illustrated in the figure). After the uptake and treatment of raw water, drinking water is distributed to the end users. Waste and stormwater are then collected from the end users and surrounding environment and treated for release. An observer may see this as one technical system for managing water, but in reality, it is segmented into the subsystems of water supply and sanitation. Such partitioning into "clean" and "dirty" water is not only administrative but fundamental and is found at all levels, from operators to research. The advantages of taking a more integrated view of the urban water cycle have been noted (*2*), but barriers to implementation remain.

Institute of Infrastructure Engineering, University Innsbruck, Technikerstrasse 13, 6020 Innsbruck, Austria. E-mail: wolfgang.rauch@uibk.ac.at

ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*