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Essay

Daddy Issues: Paternal Effects on Phenotype

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The once popular and then heretical idea that ancestral environment can affect the phenotype of future generations is coming back into vogue due to advances in the field of epigenetic inheritance. How paternal environmental conditions influence the phenotype of progeny is now a tractable question, and researchers are exploring potential mechanisms underlying such effects.

Introduction

The past few decades have seen an important expansion of our understanding of inheritance, as a wide variety of epigenetically inherited traits have been described. One implication of epigenetic inheritance systems is that they provide a potential mechanism by which parents could transfer information to their offspring about the environment that they experienced, and under certain environmental regimes, such information transfer can, in theory, be adaptive. This type of inheritance has come to be called ''Lamarckian'' inheritance after early evolutionary theorist J.B. Lamarck, although it is worth noting that both Darwin and Lamarck believed in the inheritance of acquired characters. It is increasingly appreciated in many different species that at least some environmental information can be passed on to offspring. In this Essay, I discuss a handful of recent paradigms in which ancestral environment influences phenotype in offspring, with a focus on mammals and supporting evidence from other major multicellular model systems. I will focus primarily on paternal environmental effects, as maternal effects include many cases of direct environmental action on the progeny, as in, for example, fetal alcohol syndrome. Interested readers are directed to recent reviews for additional examples (Curley et al., 2011; Jablonka and Raz, 2009; Jirtle and Skinner, 2007; Youngson and Whitelaw, 2008) and for microbial examples (Rando and Verstrepen, 2007).

Epigenetic Inheritance Models

Epigenetic inheritance, the inheritance of information beyond the DNA sequence in forms such as cytosine methylation patterns, is the likeliest mechanism by which ancestral environments could influence offspring (but see below). Epigenetic inheritance paradigms include ''programmed'' cases, such as those involved in human imprinting disorders, and cases of ''epivariation'' in which genetically identical organisms exhibit a range of phenotypes that are heritable despite not resulting from variation in DNA sequence. Imprinted genes are expressed from only one allele (maternal or paternal) in a diploid organism (Bartolomei and Ferguson-Smith, 2011). Because of this highly penetrant inheritance pattern, children with identical genotypes (such as a deletion of 15q11-13) can have wildly different phenotypes (Prader-Willi disease or Angelman's syndrome) depending on whether the deletion was transmitted from the child's mother or father. Imprinted genes thus represent a case of inheritance of ancestral genetic information.

A number of epivariable traits have been described in multiple organisms; plants in particular have been fertile ground for discovery of epivariation, with genetically well-characterized examples including paramutation in maize (Arteaga-Vazquez and Chandler, 2010), or the cytosine-methylated *clark kent* alleles of *SUPERMAN* in *Arabidopsis* (Chan et al., 2005). For example, in the best-studied case of paramutation, the presence of seven 853 bp repeats ~100 kb upstream of the *b1* locus (encoding a transcription factor that controls plant pigment levels) makes this locus ''paramutable.'' This locus can exist as the highly transcribed *B-I* allele (with resulting dark purple coloration) or the poorly transcribed *B′* allele, and these expression levels are quite stable (conversion of *B-I* to *B′* occurs at ~1% frequency, the reverse almost never occurs) despite no DNA sequence differences between the *b1* loci at these two epialleles. Thus, in these and other examples of epivariation, two plants with identical genomes can have distinct phenotypes, such as high or low pigmentation, that are stably maintained epigenetically.

In mammals, the best-studied epivariable locus is the agouti variable yellow (A^{vy}) locus; genetically identical A^{vy} mice range in color from yellow to brown, and this coloration can be passed from mother to offspring (Morgan et al., 1999; Youngson and Whitelaw, 2008). The A^{vy} locus results from an insertion of the retrotransposon IAP upstream of the *Agouti* coat coloration gene, and as with many other cases of epivariation, it is likely the presence of a ''selfish'' genetic element (here, IAP) that sensitizes this locus to epigenetic control. Decades of genetic and molecular analysis of imprinting, paramutation, and other epivariable traits have identified many of the epigenetic information pathways briefly reviewed below.

In general, epigenetic inheritance paradigms typically affect either transgenes

(Henikoff, 1998) or endogenous loci associated with repetitive DNAs (Slotkin and Martienssen, 2007). This motivates the compelling hypothesis that epigenetic inheritance mechanisms initially evolved as a way to counteract "selfish" genomic elements, and these mechanisms have since been co-opted for other aspects of transcriptional regulation. Repeat elements subject to epigenetic inheritance are often derived from widespread transposons, as in the cases of the IAP element in the A^{vy} reporter locus, the SINE-derived tandem repeats at the *FWA* locus in *Arabidopsis*, the abundance of repeats typically associated with imprinted genes in mammals, or dense repeats that drive position effect variegation in flies. However, less abundant repeats can also drive epigenetic silencing not only for transgenes, but also for endogenous cases, including the seven tandem repeats found at the paramutable *b* locus in maize (Arteaga-Vazquez and Chandler, 2010) or the *dg/dh* repeats that drive centromeric silencing in fission yeast (Grewal, 2010).

Paradigms for Inheritance of Acquired Characters: Genetic

Experiments demonstrating ancestral influence over progeny phenotype fall into two classes: those in which ancestral *genotype* affects offspring (as in cases in which heterozygous mutant animals have wild-type offspring with altered phenotypes) and those in which ancestral *environment* (such as diet) alters offspring phenotype. Ancestral genotype can influence a wide variety of phenotypes in mouse; for example, genetically identical daughters of males differing only in their Y chromosome (not inherited by daughters) may differ in traits ranging from lipid levels and bone density to anxiety-related behaviors (Nelson et al., 2010). Ancestral genotype effects on offspring phenotype can provide some insights into the mechanisms underlying transgenerational environmental effects. Specifically, many cases of ancestral genetic effects on phenotype involve genetic analysis of epigenetically variable phenotypes in which mutants induce a specific epigenetic state at a sensitive reporter locus that is maintained even after recovery of the wild-type genotype. For instance, Whitelaw and colleagues have shown in mice that males heterozygous for mutations in either *Smarca5* or *Dnmt1* can sire wild-type offspring (inheriting the wild-type *Smarca5* allele, for example, from the heterozygous father) with altered penetrance of A^{vy} expression (Chong et al., 2007).

In addition to transgenerational effects of ancestral genotype identified via analysis of epivariable reporters, more and more cases of ancestral genetic effects are uncovered without the benefit of reporter genes as increasingly detailed phenotypes are reported in appropriate breeding paradigms (Nelson et al., 2010). A prominent recent example in *C. elegans* comes from analysis of mutants in the ASH-2 H3K4 methylase complex; these mutants can give rise to approximately three generations of progeny that exhibit extended life span despite the fact that the relevant mutation has segregated away (Greer et al., 2011). Conversely, *C. elegans* mutants lacking the H3K4 demethylase LSD1 exhibit progressive sterility over 20–30 generations, with H3K4me2 levels accumulating over time (Katz et al., 2009); in this case, mutant animals seem to "remember" their wild-type ancestry for ~20 generations before succumbing to the effects of the mutation. Interestingly, in these and many other cases of transgenerational genotypic effects, even when the reported phenotype does not rely on a sensitized epivariable reporter gene, the memorable ancestral genotype involves an alteration in a regulator of one of the major epigenetic information carriers—small RNAs, chromatin state, or cytosine methylation (see below).

Paradigms for Inheritance of Acquired Characters: Environmental

A large number of studies report that the environment experienced by parents can affect offspring who never experience that environment. Rather than attempt a comprehensive listing of such studies, I focus on two general types of environment that appear to affect descendants in various organisms: stress/toxins and nutrient availability.

Stressful environments, including social defeat (Dietz et al., 2011), DNA-damaging stresses (Hauser et al., 2011), and environmental toxicants, have a multitude of effects on future generations even once the stress has passed. Most famously, injection of high concentrations of the endocrine disruptor vinclozolin into pregnant female rats results in diminished fertility over three to four generations of offspring, with phenotypes including increased testicular apoptosis and altered behaviors being transmitted through the male germline (Anway et al., 2005; Jirtle and Skinner, 2007). More recently, it was found in flies that stressing early embryos with heat shock or osmotic stress results in derepression of heterochromatin, as assayed by the eye pigment reporter of position effect variegation (PEV). PEV derepression occurred not only in the stressed animals, but also in their progeny, and could be transmitted either maternally or paternally (Seong et al., 2011). Curiously, transmission of derepressed heterochromatin affected the PEV reporter in *trans*, as stressed males were crossed to control females carrying an X-linked *white* reporter, and male offspring of this cross exhibited derepression of the reporter derived solely from the unstressed females (see also below).

A great deal of evidence links ancestral dietary conditions to metabolic phenotypes in offspring. In humans, epidemiological studies link maternal undernutrition with increased risk of type 2 diabetes and obesity in children, an observation that motivates the famous "Barker hypothesis" (Hales and Barker, 2001) or "thrifty phenotype hypothesis." This holds, essentially, that, if your parents tell you that you're going to go hungry, it makes sense to hoard calories, a trait that may be maladaptive if conditions of plenty return. Supporting this idea, Dutch children who were subject to in utero starvation during the Hunger Winter of 1944–1945 suffer increased rates of diabetes, cardiovascular disease, and obesity later in life. Whereas these and a multitude of rodent studies (Li et al., 2011) show clear maternal effects of food availability on offspring, related paternal effects have also been discovered. Specifically, epidemiological data from human populations link food availability in paternal grandparents to obesity and cardiovascular disease two generations later (Kaati et al., 2002; Pembrey et al., 2006). Here, transmission of disease risk is sex specific: grandson's

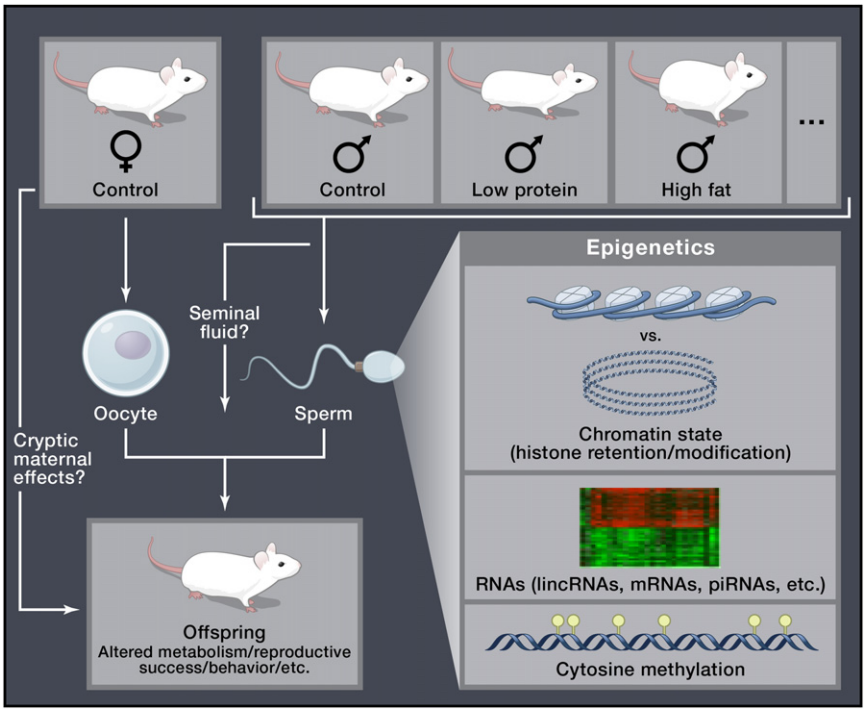

Figure 1. Potential Mechanisms Underlying Paternal Environmental Effects on Offspring Phenotype

In typical rodent models for paternal effects, male littermates are either split to control conditions or are subject to a variety of environmental conditions, including dietary alterations, social stresses, toxins, and so forth. After mating to control females, phenotypic alterations are often observed in offspring in these paradigms. Illustrated here are a number of candidate mechanisms underlying such paternal effects, including (1) alterations in the sperm epigenome, (2) effects of seminal fluid on offspring, and (3) so-called "cryptic maternal effects" in which females judge males and alter how they care for offspring accordingly.

relative risk was linked to the diet of his paternal grandfather, but not paternal grandmother, whereas granddaughter's risk was conversely only associated with her paternal grandmothers' diet. Curiously, in both cases, if the relevant grandparent experienced poor food access in early adulthood (~19 years old), the grandchild had an increased mortality risk, whereas in early adolescence (~10 years old), inadequate food was instead linked to decreased disease risk in grandchildren.

Rodent studies confirm that paternal dietary conditions can affect offspring metabolism and avoid pitfalls in analysis associated with the outbred nature of human populations. Male mice subjected to preconception fasting sire offspring with altered glucose metabolism (Anderson et al., 2006), whereas male rats chronically consuming a high-fat diet sire daughters with a number of pancreatic phenotypes, including decreased glucose tolerance, and decreased numbers of islet cells (Ng et al., 2010). In our lab, we found that male mice consuming a low-protein diet (from weaning to sexual maturity) fathered offspring with decreased hepatic levels of cholesterol esters and altered hepatic expression of lipid/cholesterol biosynthesis genes (Carone et al., 2010). Similar results were reported by Ferguson-Smith and colleagues, who showed that lipid/cholesterol gene expression (Radford et al., 2012) and glucose tolerance were altered in embryos whose fathers had been subject to undernutrition while in utero. In most of these cases, analysis focused on the progeny of the treated male. It will be interesting in the future to extend paternal dietary studies to additional generations of progeny.

These and other studies make the compelling case that a male's environment, either during development or during adulthood, can affect a variety of phenotypes in his children. All of this, of course, prompts the question of how it all works.

Is Paternal Environmental Information Carried in Sperm?

It is a natural hypothesis that paternal environmental effects are transmitted via changes in one of the several sperm "epigenomes" (Figure 1). However, other information carriers exist by which fathers can influence progeny phenotypes. First, in human populations, it is eminently plausible that paternal environment selects for particular sperm haploid genomes, e.g., that the environment skews the genotype distribution in an ejaculate. This is one of the motivations for using inbred animal models for transgenerational studies, as all sperm are in principle genetically identical. Nonetheless, it is still possible that the environment alters the sperm genotype in a reproducible way via directed DNA editing or transposon-mediated mutagenesis.

Cultural inheritance mechanisms may also play a role in transgenerational inheritance. Many examples of maternal cultural inheritance have been described; for example, food preferences can be transmitted to young mice via maternal milk (Avital and Jablonka, 2000). In rats, maternal care (the extent of maternal grooming and nursing) affects cytosine methylation and gene expression in the brains of offspring (Fish et al., 2004). As offspring age, these alterations affect the quality of maternal care that these animals later provide to their young, thus propagating the caring/uncaring maternal phenotype over generations. In maternal effect paradigms, cultural inheritance can be ruled out via oocyte transfer or cross-fostering experiments (Morgan et al., 1999).

However, in most paternal environmental effects on offspring, males are unlikely to exert any direct influence over progeny; males are removed shortly after mating with females in our lab and in many of the related studies. Nonetheless, males can impact maternal care and thereby influence embryonic development or cultural inheritance indirectly, as documented most extensively in various birds (Curley et al., 2011). A clear example is found in the Gouldian finch, in which simply painting the head of the male different colors can alter a female's

investment (egg size, number, and gender) in their offspring, an outcome proposed to result from the female's perception of the male's compatibility (Pryke and Griffith, 2009).

Sperm are not the only potentially relevant components of an ejaculate that might influence offspring phenotype. Seminal fluid can alter female postcopulatory behaviors from willingness to remate to feeding behavior in flies (Avila et al., 2011) and affect uterine inflammation, progesterone synthesis, and the kinetics of embryo development in mammals. The extent to which seminal fluid contents are influenced by diet or stress, and how this impacts offspring phenotypes, is unclear at present. Finally, even basic aspects of sperm biology such as sperm motility can be affected by paternal conditions and could potentially affect offspring phenotype by, for example, altering the position within the fallopian tube where fertilization occurs.

It is clear that, even when males do not directly interact with their offspring, there are nonetheless many potential ways, beyond the sperm epigenome, that males could plausibly influence offspring. Ruling in/out such nongametic information carriers is challenging, and experimental paradigms for doing so vary depending on the organism in question. In *C. elegans*, the ability of hermaphrodites to mate with males or with themselves allowed Alcazar and Fire to use a successive mating protocol to make the case that the factors required for paternal transmission of RNAi-mediated silencing (see below) are located in sperm (Alcazar et al., 2008). In mammals, artificial insemination or in vitro fertilization can eliminate maternal judgment of fathers or seminal fluid-based influences. However, epigenetic alterations associated with superovulation or with embryo culture (Chason et al., 2011) may affect transmission of relevant epigenetic information in IVF experiments, so results must be interpreted with caution.

Epigenetic Information Carriers in Sperm

Epigenetic inheritance remains the likeliest candidate to carry paternal information to offspring. Study of the mechanisms underlying imprinting, PEV, epivariation in plants, and other epigenetic phenomena have uncovered three major classes of potential epigenetic information carrier: cytosine methylation, chromatin structure, and RNA.

Cytosine Methylation

A subset of genomic cytosines is methylated at the C5 position in a number of species. In mammals, cytosine methylation primarily occurs in the context of the CpG dinucleotide, whereas in plants, non-CpG cytosines can also be methylated. Cytosine methylation is a heritable epigenetic modification implicated in many of the best-established epigenetic inheritance paradigms, although it is worth noting that major model organisms such as worms and flies have perfectly functional epigenetic inheritance despite little to no cytosine methylation. Epigenetic cytosine methylation states not only include those that are programmed and largely invariant, as observed at the differentially methylated regions involved in imprinting, but also methylation events that are epigenetically variable in populations (Feng et al., 2010). Cytosine methylation is involved in epivariation at the *FWA* and *SUPERMAN* loci (and many others) in *Arabidopsis* and at $Axin^{Fu}$ and A^{vy} in mouse. In such cases, animals or plants with high levels of methylation at a given locus tend to have offspring with high methylation, and likewise for low methylation levels.

How are paternal cytosine methylation patterns maintained? Soon after fertilization, the vast majority of methylcytosine in sperm is converted by the Tet3 enzyme to hydroxymethylcytosine, which appears to be lost by dilution during replication, thereby effectively erasing cytosine methylation patterns (Wu and Zhang, 2011). Conversely, maternal cytosine methylation is protected from hydroxylation by the PGC7/Dppa3/Stella protein and can therefore effectively be maintained. Despite the widespread hydroxylation of the paternal methylome, a subset of paternal cytosine methylation marks is maintained, including at some imprinted genes. Recent studies suggest that PGC7/Dppa3/Stella, which protects the maternal genome from demethylation, is targeted to the genome via binding to the heterochromatic histone mark H3K9me2 (Nakamura et al., 2012). Intriguingly, H3K9me2 was found at several paternally methylated imprinted regions in sperm, raising the possibility that this histone mark signals special windows of the paternal genome where methylation status will be maintained.

Chromatin Structure

Eukaryotic genomes are packaged into a nucleoprotein complex known as chromatin. Germ cells exhibit highly unusual chromatin states that are vastly different from other cell types (Ooi and Henikoff, 2007). In mammals, most histone proteins are lost during spermatogenesis, eventually replaced by protamines. However, not all histones are lost, and genes expressed early during development may preferentially retain histones in sperm (Brykczynska et al., 2010; Hammoud et al., 2009). After fertilization, the sperm genome is rapidly stripped of protamines and most (but not all) histones and is globally incorporated into H3.3-containing nucleosomes (Ooi and Henikoff, 2007). Evidence that gametic chromatin states may be heritable comes from transgenerational genetic effects of chromatin mutants (Chong et al., 2007; Greer et al., 2011) and the transgenerational effects of heat shock on heterochromatin in flies (Seong et al., 2011), as well as the observation (noted above) that inheritance of cytosine methylation may depend on the coincident occurrence of methylcytosine with H3K9me2-marked histones. It is nonetheless important to be aware that phenotypic effects on offspring of chromatin-related mutants or of stress may not result directly from chromatin changes in sperm, as other epigenetic information such as RNA abundance (for example) may be altered in sperm from chromatin-related mutant animals.

RNA Populations

The germ cells of many different organisms carry RNAs that can affect the phenotype of offspring. Most famously, induction of RNA interference (RNAi) in *C. elegans* (Fire et al., 1998) results in heritable RNA-mediated gene silencing for approximately four to five generations. Silencing induced by RNAi can be paternally inherited in worms, and elegant genetic analyses show that the silencing factor is located in sperm and is likely to be diffusible, as it can silence chromosomal targets in *trans* (Alcazar et al., 2008; Grishok et al., 2000). Examples of functional RNAs in gametes include small

"antitransposon" piwi-interacting RNAs (piRNAs) in fly oocytes and in pollen (Ghildiyal and Zamore, 2009) and functional mRNAs packaged in pollen that will be translated in the early *Arabidopsis* embryo. In mammals, sperm carry both long RNAs as well as small RNAs, including microRNAs and piRNAs. Small maternal RNAs can be stable for several cell divisions and continue to play roles in gene and transposon regulation (Suh and Blelloch, 2011). Conversely, paternal piRNAs are not sufficient to direct silencing of transposons in *Drosophila* hybrid dysgenesis systems, and most paternal mRNAs are degraded after fertilization in mammals. This stands in contrast to the likelihood that sperm-delivered small RNAs are the transmissible epigenetic signal in *C. elegans* RNAi (Alcazar et al., 2008; Grishok et al., 2000). Thus, although there is some evidence that paternally transmitted RNAs could potentially affect early embryonic development or later phenotypes in mammals (Rassoulzadegan and Cuzin, 2010), it is currently unknown what features distinguish RNAs that survive early degradation and have later functional consequences.

Other Potential Epigenetic Carriers

Additional epigenetic information carriers are plausible. For instance, prion states of numerous proteins are stably heritable both through mitosis and through meiosis in budding yeast. Although in mammals prion-mediated diseases such as Creutzfeldt-Jakob disease do not seem to be transmitted vertically, a number of other proteins that are capable of forming (potentially nonpathogenic) amyloids in vitro (von Horsten et al., 2007) have been identified associated with the sperm acrosome (Guyonnet et al., 2012). Beyond prions, other proteins such as transcription factors or the abundant protamines (which, like histones, are subject to a wide variety of covalent modifications) present in sperm could conceivably alter the phenotype of offspring.

Epigenetic Crosstalk

Further complicating matters, every one of the better-understood epigenetic information carriers exhibits crosstalk with every one of the other carriers. Cytosine modifications directly affect nucleosome positioning and recruit chromatin-modifying complexes, and conversely histone modifications can affect recruitment of cytosine methylases and demethylases. Small RNAs, including short interfering RNAs (siRNAs) and piRNAs, and long RNAs, such as long intergenic noncoding RNAs (lincRNAs), can direct histone modifications and cytosine methylation. Finally, chromatin structure and DNA modifications affect transcription of small RNA and lincRNA-containing loci. The importance of such crosstalk is that analysis of epigenetic marks in offspring, as carried out in multiple studies, might well report on the eventual downstream effects of some original and perhaps long-erased epigenetic perturbation.

Multiple Information Carriers versus an Environmental Quality Metric

How much environmental information can mammalian sperm carry? Do sperm carry information about tens or hundreds of important environmental conditions (integrated caloric input, presence/absence of various environmental toxicants, social status, etc.), or do diverse environmental conditions simply alter sperm "quality," which then affects many different downstream phenotypes? Perhaps counterintuitively, the simplest hypothesis is that epigenetic information carriers enable high-bandwidth transmission of environmental information. This is motivated by the abundance of potentially epigenetic loci (the ~20 million CpGs in a human haploid genome could each potentially transmit a "bit" of information in sperm). In addition, stable epivariable phenotypes can often be separated from one another in meiosis, as observed for the *MePAI2* and *MePAI3* epialleles in *Arabidopsis* (Bender and Fink, 1995), indicating that these two epialleles are not sensitive target loci responding to alterations in some unlinked *trans*-acting regulator of global methylation.

However, in the case of transgenerational inheritance of environmental information, it is unclear how many distinct phenotypes can be influenced. Most studies in mammals have focused on different phenotypes (metabolism is studied in response to paternal diet, behavior is studied in response to paternal social defeat, etc.), but when checked, it often turns out that overlapping phenotypes can be seen in response to distinct paternal treatments. For example, not only do endocrine disruptors affect future reproductive success of males, but reproductive success can also respond to ancestral exposure to high-fat diet in utero. Moreover, altering early embryonic development can have effects similar to those observed in paternal environmental exposure paradigms. For instance, humans born after in vitro fertilization exhibit altered glucose tolerance (van Montfoort et al., 2012), and brief in vitro culture of mouse embryos results in increased expression of the epigenetically sensitive A^{vy} reporter gene and could alter expression of imprinted genes.

Another hint that sperm may transmit some overall stress measure is that several cases of transgenerational effects turn out to affect epigenetically sensitive reporter genes that were not present in the parent subject to genetic or environmental stressors. As described above, heat shock of early fly embryos (male) can affect silencing of the *white* reporter in offspring even when this reporter is only inherited from an unstressed mother. Similarly, Whitelaw and colleagues found that certain heterozygous male mutants (*Snf2h* or *Dnmt1*) can sire wild-type offspring with altered expression of the A^{vy} reporter locus (Chong et al., 2007), even when the reporter locus A^{vy} was transmitted maternally. The fact that paternal genotype/environment can affect reporter genes in *trans* shows that, in these cases, such effects are not purely locus specific (e.g., sperm-specific changes at the chromosomal A^{vy} locus) but might instead affect: (1) overall assembly/maintenance of heterochromatin in the early embryo or (2) silencing of specific widespread repeat elements (e.g., IAP versus LINE, etc.) that could in turn affect dispersed targets. Consistent with the former hypothesis, many epigenetic inheritance paradigms, including genetic effects on PEV reporters and paternal effects of high-fat diet, exhibit differences between male and female offspring, a result sometimes hypothesized to result from X chromosome copy number acting as a "sink" for epigenetic factors in offspring. The identity of a hypothetical sperm-carried regulator that affects global heterochromatin levels

(for instance) in the embryo remains mysterious.

Together, these results provide some support to a "sick sperm" hypothesis, whereby multiple paternal stressors might affect some aspect of sperm maturation (motility, etc.), thereby influencing future phenotype via effects on preimplantation development. Arguing against this hypothesis are multiple lines of evidence; for instance, paternal environmental effects, such as transgenerational effects of heat shock in flies, have been reported in animals such as flies and worms in which embryo development is quite different from that in mammals. Overall, it seems fairly likely that differing paternal environments are capable of influencing a number of quite distinct phenotypes in offspring. But in mammals, this remains to be conclusively shown.

Epigenetic Contributions to Human Disease

Epigenetic defects are increasingly understood to contribute to human disease. Beyond epigenetic changes that occur during an individual's life span (e.g., in oncogenesis), there is mounting evidence that ancestral environment can affect current disease risk in humans. Most convincingly, ancestral nutritional status has been linked to metabolic disease in children and grandchildren (Hales and Barker, 2001; Kaati et al., 2002; Pembrey et al., 2006). These and other findings strongly suggest that future epidemiological studies will need to address not only whether parents experienced a particular environment, but also when this experience occurred relative to conception. In other words, perhaps the question is not *whether* a patient's father drinks alcohol, but *when* he started relative to when the patient was conceived.

Such considerations call for a rethinking of studies of complex diseases with a heritable component, such as diabetes, schizophrenia, or alcoholism. Indeed, a burgeoning field of "epigenetic epidemiology" seeks to uncover epigenetic marks that might potentially explain missing heritability in complex diseases (Rakyan et al., 2011), although most such efforts focus on histone or DNA marks in affected and unaffected cohorts (e.g., in the current generation), thus lumping together marks that stem from parental environments with those stemming from a person's current lifestyle. More specific to parental effects, future environmental exposure histories will need to include parental exposure histories as well as exposure histories of the individuals studied so as to disentangle induced epigenetic effects from the currently sought genetic and environmental causes of complex diseases.

Conclusions and Perspective

Given the bulk of experimental evidence from many different paradigms, it is clear that paternal environmental conditions can affect the phenotypes of offspring in multicellular organisms. Extensive genetic and molecular evidence supports a role for interconnected epigenetic information carriers such as RNAs, chromatin state, and DNA modifications in transgenerational inheritance of epivariable phenotypes. In most cases of transgenerational genetic/environmental inheritance, it is not yet clear how the relevant information is carried from parent to child, but epigenetic information is likely to be relevant for many or most such cases. The coming years hold great promise for untangling the mysteries of this exciting class of phenomena.

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