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Epigenetic inheritance of metabolic state

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As the incidence of complex metabolic disease increases in developed countries, so too does the need to understand the causes and risk factors for these disorders. In addition to the well-known contribution of genetics and environment to metabolic dysfunction, many studies have demonstrated that a significant degree of non-genetic heritable risk can be transmitted from parents to offspring over multiple generations. Understanding the mechanisms by which this occurs could change how we study and treat complex metabolic disorders. In this review, we summarize recent advances in this field utilizing *Drosophila*, mice, and humans, and propose potential molecular mechanisms that underlie the transgenerational inheritance of metabolic state.

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Introduction

The incidence of complex metabolic disorders has increased at an alarming rate over the past two decades. Approximately one-third of adults and one-sixth of children in the United States fit the medical definition of obesity [1]. The incidence of type 2 diabetes is also on the rise, with 7% of American adults diagnosed and likely more undiagnosed [2]. Medical costs of these disorders are approaching $200 billion each year [2]. A major field of current biomedical research is therefore focused on understanding the progression of complex metabolic disorders and developing potential treatments and preventative strategies.

Both genetic and environmental components contribute to the regulation of metabolism and the risk of metabolic disease. A large percentage of this risk is heritable, but many of the responsible genetic factors remain unidentified. In the past two decades, researchers have explored gene–environment interactions as a potential source of at least some of this unknown risk. These studies have demonstrated an unexpected influence of both parental and developmental nutritional environment on adult metabolic health. What is more, there is now a great deal of evidence suggesting that these induced changes in metabolic state can be inherited for several generations. The transgenerational inheritance of metabolic phenotypes could explain some of the missing heritability in complex disorders and change how we think about metabolic health [3].

Evidence for inheritance of metabolic state in humans

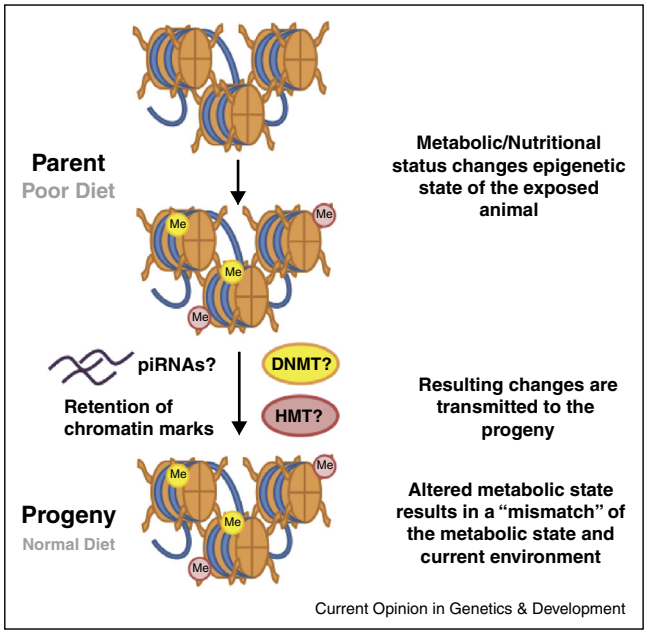
The role of developmental and parental environment in shaping adult metabolism has been strikingly demonstrated in several human studies that followed individuals exposed to extreme nutrient deprivation during gestation. The most famous of these derives from the Dutch Hunger Winter, a period of severe caloric restriction at the end of World War II in German-occupied Holland. Compared to control siblings, individuals exposed to the famine during gestation were at increased risk for metabolic disorders such as diabetes and obesity [4–6] as well as cardiac-related disorders such as hypertension [4]. Similar observations were made in a study focusing on the Chinese famine (1959–1961). Furthermore, this risk was at its highest when gestational famine exposure was followed by consumption of a high calorie, western-style diet later in life, as compared to those who continued to consume a lower calorie diet [7].

The common variable in these and other studies is the contrast between gestational and adult nutritional environments. This research has led to a unifying model for the effects of parental diet on the metabolic state of offspring (Figure 1). Exposure to a change in nutritional environment sets up a metabolic state in the parents that is best suited to that altered environment, and this state is transmitted to the offspring through epigenetic marks in the parental gametes or developing fetus. If, however, progeny nutrition differs from that of the parents, their metabolism will no longer be adapted to the environment. This kind of 'mismatch' could be a source of altered risk for developing complex metabolic disorders [8].

Rodent models for studying the inheritance of metabolic state

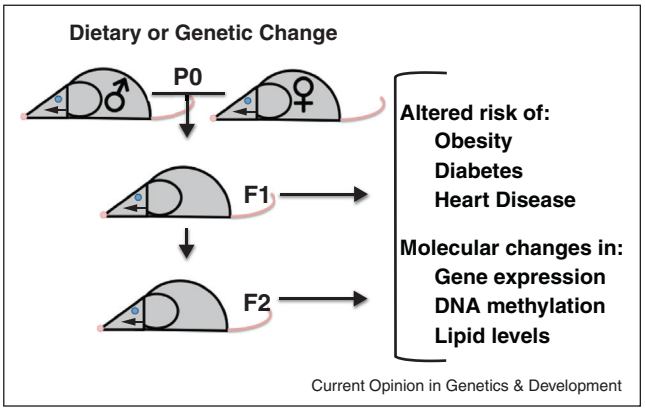
Many studies have used rodents to better define the mechanisms underlying the inheritance of metabolic state. In the most commonly used paradigm, a female mouse or rat is fed a low protein or low calorie diet during pregnancy. After birth, the mother and pups are switched to a relatively normal control diet for the duration of their

Figure 1



Model for the epigenetic transmission of metabolic state. Parents are exposed to a change in nutritional environment that alters the epigenetic state of their chromatin, both in somatic tissues and in the germline. These changes are transmitted to the progeny, setting up a metabolic state that is best adapted to the parental environment. If the environment of the progeny deviates from that of the parents, their metabolic state is no longer adapted to their environment and disease may occur. Changes in chromatin state are represented by DNA and histone methylation, but can include other post-translational modifications such as acetylation or ubiquitylation. Other mechanisms, such as piRNAs, may also be involved in transmission.

Figure 2



Rodent paradigm for the transgenerational inheritance of metabolic state. Either the maternal or paternal parent is exposed to a genetic or dietary change that alters its physiology. This challenge is removed in the next generation. Regardless of which parent is affected or the mode of alteration used, the F1 and F2 progeny have altered metabolic disease risks and molecular readouts for metabolites and gene expression.

lives [8,9,10•,11•,12–17]. While the results vary depending on genetic background and the phenotypic outputs measured, they are remarkably similar to the previously discussed human studies. Mice exposed to a low protein diet during gestation have an increased risk of obesity and diabetes [16], cardiovascular disease [14], and other defects [17] (Figure 2). These physiological changes correlate with molecular changes in hepatic gene expression [9,11•], increased circulating triglyceride and fatty acid levels [10•], an altered distribution of these metabolites throughout the body [13], and changes in DNA methylation [11•]. In the few cases where it has been measured, physiological and molecular changes extend through several subsequent generations [9,16]. In one particular set of studies, expression increased for two metabolic transcriptional regulators, PPARα and glucocorticoid receptor [10,11•]. There was a correlated decrease in the level of DNA methylation at the promoters of both genes, and supplementation of the maternal diet with folate rescued both phenotypes. Folate is an important dietary precursor to the cofactors required for methyl transfer reactions, suggesting that there is a functional link between the epigenetic marks on DNA and chromatin and the expression of metabolically relevant genes [11•]. Recent experiments using a folate pathway mutant confirm the importance of normal folate metabolism in epigenetic regulation during development for up to two generations after loss of the mutation, supporting the model that methylation patterns contribute to transgenerational inheritance [18].

Interpretation of experiments that depend on changes in maternal physiology is complicated by the confounding variable of maternal environment during fetal development. As a result, paternal models have been developed that alter nutritional or metabolic state. Importantly, this work largely agrees with the maternal studies and suggests that both males and females can contribute to metabolic programming. In a study that closely parallels the most commonly used maternal paradigm, male mice were fed a low protein diet before mating with females fed the control diet [19••]. Daughters have significantly altered liver lipids compared to controls, including increased triglycerides and saturated free fatty acids. Metabolic gene expression and DNA methylation patterns are also misregulated. Notably, many of the differentially expressed genes are known targets of metabolic regulators, including PPARα and SREBP. Even more strikingly, expression of PPARα itself is downregulated in daughter livers, correlating with an increase in DNA methylation upstream from PPARα, albeit at a distal site whose influence over PPARα expression is unclear [19••]. Other examples corroborate the ability of paternal environment to influence metabolism in the next generation. Offspring from fathers fed a high fat diet display

decreased glucose tolerance and altered islet gene expression [20,21]. Genetically altering paternal metabolism can also lead to differential molecular and physiological responses in wild-type progeny for several generations [3,22]. For example, an allele in the *Obrq2A* locus is associated with reduced body weight, levels of circulating glucose and insulin, and food intake in fathers. After two or three generations of outcrossing, the progeny continue to display decreased food intake and body weight [22]. The clear ability of paternal metabolic state to influence offspring metabolism further supports a model where epigenetic changes in the gametes can lead to heritable changes in offspring metabolism.

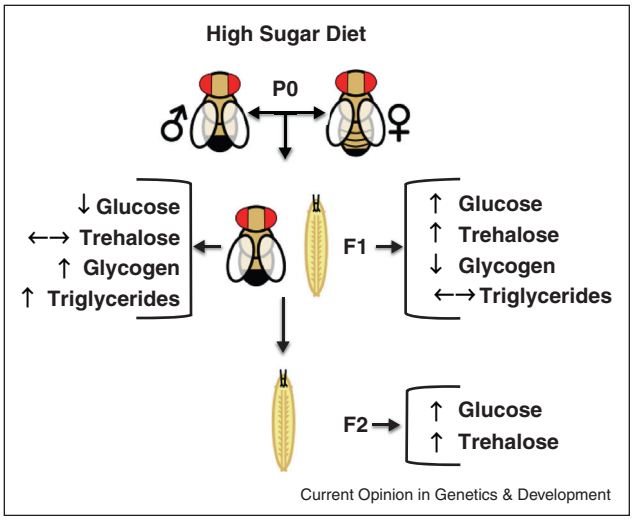
Evidence for the inheritance of metabolic state in *Drosophila*

While great strides have been made using rodent models, the results remain correlative and no specific molecular mechanisms have been discovered. One possible solution to advance the field is to take the paradigm into a simpler model system, specifically the fruit fly *Drosophila*. Because many epigenetic pathways have been extensively described in fruit flies, a vast number of tools are already available for these studies. Two papers published in the last year have demonstrated that this phenomenon is conserved in *Drosophila*, providing a foundation for further research in this system [23••,24••].

In one paper [23••], a number of lines derived from single females in a wild population, referred to as iso-female lines, were subjected to either low or high protein relative to sugar, isocaloric diets during larval development. The F1 progeny were subsequently reared on a standard diet. In most of the lines, those progeny whose parents were exposed to the low protein diet have increased glycogen and decreased triglyceride levels as compared to progeny of parents exposed to the high protein diet. Interestingly, these changes are of different magnitudes in different strains, from nearly nonexistent to a two or threefold difference, with a change in the opposite direction in one strain [23••]. The results of this study demonstrate that, like mammals, parental diet can influence progeny metabolism in *Drosophila*. They also show the importance of a controlled and uniform genetic background in order to reproducibly and accurately record these responses.

The second study focuses on responses to a high sugar diet fed to adult females [24••]. They record slight increases in circulating sugars and decreased glycogen storage in larvae from exposed mothers. A different response, however, is observed when these progeny are followed to adulthood, with decreased glucose and increased glycogen stores in adult males, the opposite of the larval phenotype (Figure 3). Challenge of adult progeny with the high sugar diet exacerbates these phenotypes and leads to more dramatic increases in triglyceride and trehalose levels than those seen in control

Figure 3



Drosophila display transgenerational inheritance of metabolic state. Female flies are fed a high sugar diet and their offspring are raised on a normal diet [24••]. F1 larvae have increased glucose and trehalose levels and decreased glycogen levels, while F1 adults show an opposite effect. Adults also display increased triglycerides as compared to controls. Larval changes in glucose and trehalose can be transmitted to the F2 generation.

progeny, indicating a propensity in these progeny toward diet-induced obesity as seen in mammalian studies. Interestingly, increased circulating sugars persist in larval stages through the F2 generation. A number of lipid and carbohydrate metabolic genes also change their expression in the F1 progeny. Unlike metabolite levels, however, some of these changes are not exacerbated when the progeny are challenged with the high sugar diet. On the contrary, for some genes, high sugar challenge in progeny of high sugar-fed mothers equilibrates expression to control levels of low sugar-fed progeny from low sugar-fed parents [24••]. Although not precisely correlated, these 'rescued' changes in gene expression support the model that progeny metabolism is adapted to the parental environment. Taken together, these two papers establish *Drosophila* as a valid model for studies of the epigenetic inheritance of metabolic state, and provide a foundation for future studies into the mechanisms that underlie this regulation.

Potential epigenetic mechanisms

Although several potential epigenetic processes have been proposed to link parental environment with adult progeny metabolism, molecular mechanisms remain to be identified. The evidence that correlates promoter DNA methylation with metabolic gene expression in rodents supports the model that altering DNA methylation levels or locations within the genome could be responsible for the resulting molecular changes [11•,19••]. Attempts to prove this hypothesis, however, have unfortunately fallen

short thus far. In the male low protein diet study, correlations were identified between daughter DNA methylation levels and gene expression levels. When male sperm were examined, however, there was no correlation between DNA methylation changes observed in the sperm and the daughters [19••].

Direct changes to chromatin can also take the form of histone modifications such as methylation or acetylation. Because these chromatin marks tend to be more dynamic than DNA methylation, their levels have not been examined as closely [25]. New evidence, however, suggests potential molecular mechanisms by which histone modifications can be retained through cell division. During mitosis, while the actual histone modifications themselves are often lost, some of the modifiers responsible for these changes, such as the Polycomb and Trithorax complexes, may be retained at the sites [26,27]. This type of mechanism could contribute to histone modifications that are inherited through meiotic divisions.

Both histone modifications and DNA methylation undergo drastic changes during embryonic and germ cell development [28–30], such that neither type of mark may be sufficiently retained to transmit the necessary information across generations. Alternative mechanisms involving small non-coding RNAs (ncRNAs) have therefore been proposed based on epigenetic studies in *Drosophila* and *Caenorhabditis elegans*. One example of small ncRNAs directing heritable changes in gene expression is paramutation, which is a form of epigenetic inheritance whereby one allele at a gene locus is capable of inducing a non-genetic change in the paired allele that can be inherited through meiotic divisions [31••]. An artificial model of paramutation in *Drosophila* utilizes a tandem array of inserted *lacZ* transgenes from which the enzyme β-galactosidase is expressed. Some of these arrays are capable of silencing single *lacZ* transgenes at different loci within the genome [32]. It has been recently shown that the production of piRNAs, small RNAs that are important for transposon silencing [33], is required for this effect [31••]. The piRNA pathway is also involved with the epigenetic repression of other, naturally present transgenes in *Drosophila* [34]. In addition, piRNAs appear to contribute to the transmission of RNAi across generations in *C. elegans* [35•,36•,37•]. These roles for piRNAs in epigenetic transmission of information for multiple generations in several models and systems suggest that they may be involved in the inheritance of metabolic state. The small ncRNA hypothesis is supported by changes in miRNA expression recorded in mouse testes after feeding a high fat diet [21].

TThe development of methods to characterize the genome-wide patterns of transcription and epigenetic marks provides a powerful new approach for determining the mechanisms that underlie the transgenerational inheritance of metabolic state. These studies may explain some of the missing heritability in genome-wide association studies of diabetes and obesity. They may also provide a new therapeutic basis for understanding how effects on parental metabolism can impact the metabolic health of offspring, providing new and important insights for early intervention and treatment of complex metabolic diseases.

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