

PERSPECTIVES

Nutrition meets heredity: a case of RNA-mediated transmission of acquired characters

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Abstract

RNA-based inheritance provides a reasonable hypothesis to explain multigenerational maintenance of the disease in the progeny of either a male or female parent suffering from the metabolic syndrome (obesity and type 2 diabetes) induced by abnormal diet. Although, it is still difficult to formulate a complete rational mechanism, study of inheritance is a most direct way to learn about the epigenetic control of gene expression and we wished to summarised our current approach along this line.

Key words: sperm; non coding RNA; diet; epigenetic heredity; Mice

A living organism is continuously undergoing changes, from the egg to the adult life: diet, climate, and for humans, lifestyle, sport, education, culture. Could part of these ‘acquired characters’ be transmitted to the offspring? Asking this question is now technically possible by experiments performed on model organisms.

In addition to the Mendelian inheritance of structural information carried by genomic DNA, evidence now accumulating suggests a distinct form of heredity based on epigenetic control by noncoding RNA molecules. Unlike DNA-based inheritance, this heredity may account for the transgenerational maintenance of acquired characters, changes generated by the environment during the life of the genitor. Together with other heritable traits, RNA-based inheritance provides a reasonable hypothesis to explain the multigenerational maintenance of the disease in the progeny of either a male or female parent suffering from the metabolic syndrome (obesity and Type 2 diabetes) induced by abnormal diets. Although it is still difficult to generate comprehensive mechanistic models, experimental systems are available to evaluate RNA-mediated transgenerational determination.

Transgenerational transmission, both maternal and paternal, of a variety of diseases as been well documented. The mechanisms underlying these instances of non-Mendelian heredity are currently under study, especially those of father to offspring transmission. It is obvious that the physiological state of the mother impacts on the development of the offspring, thus making the analysis more complex. Since the basic fact of transgenerational transfer is identically observed from mothers and fathers, one may as a first approach hypothesize that paternal contribution is limited to the content of one spermatozoon.

Inherited Effects of Paternal Diet on Offspring Obesity and Metabolic Syndrome in Model Organisms

Prompted by the current worldwide epidemics of obesity and associated disorders [1] and by clinical observations indicating paternal transmission of the disease [2, 3], experimental models have been developed. Unlike clinical observations, these

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models include defined genotypes and controlled dietary conditions in a variety of organisms in which metabolic pathways are highly conserved, from *Drosophila* to mammals. In *Drosophila*, a high-sugar diet leads to obesity and as little as two days of dietary modification in fathers triggers the development of obesity in offspring [4]. The high efficiency of establishment and transmission of the disease clearly exclude a mutational mechanism. In mouse models, transgenerational signal(s) carry the memory of past events to create a new phenotype. It was in mice that RNAs were identified as candidate vectors, by a direct approach involving microinjection into one-cell embryos [5].

Several rodent models have been analyzed for their responses to dietary conditions, with the general conclusion that obesity and Type 2 diabetes are generated by hyperlipidic diets [6–10]. The results depend, however, on the genetic background. Unlike the ‘classical’ genetic analysis that would show that mutations in several loci reproducibly result in obesity, complex and still largely undefined differences between inbred genotypes are associated with distinct responses to diet, ranging from limited obesity and normal glucose regulation to full obesity and diabetes.

At the molecular level, effects of the diet on gene activity have been reported not only in the expected organs such as intestine, liver and pancreas but also in the germ line. Patterns of DNA methylation and chromatin proteins of target organs (liver, pancreas) and gametes (testis and sperm cells of the affected males) were found to be modified, corresponding to distinct gene expression profiles [11, 12]. The same syndrome was observed in the abnormally fed animals and in progenies generated with healthy partners and grown on normal diet.

Epigenetic Signaling

Possible mechanisms underlying the hereditary maintenance of a variant pattern of gene expression have been extensively discussed [11, 13–15]. Possible roles of chromatin modification and changes in DNA methylation as transgenerational signals of the modified expression were hypothesized, with, however, the restriction that most of the chromatin structure and the DNA methylome of the sick parent undergo massive reprogramming during germ cell differentiation and in the early embryo. Chromatin changes had been considered as candidates for transgenerational determination after the discovery that some of them were maintained in sperm, but further studies indicated that these few instances of maintenance did not correspond to coding regions [16]. While we cannot exclude a contributing role of some of these modified chromatin structures as specific signals that would contribute to recapitulate parts of the process in successive generations, sperm RNAs still appear today as the prime candidates.

RNA-Mediated Transmission of Paternal Signals

Whole genome sequencing established that the largest part of our genomes does not encode proteins but rather ncRNAs (non-protein-coding RNAs) [17]. Among these, miRNAs and their roles in nutritional control and associated pathologies have been the most thoroughly investigated [18]. Whether specific RNAs are responsible for the transgenerational establishment of the diet-induced disease is still a matter of speculation, but this more and more appears as a reasonable hypothesis.

Such a role of non-coding RNAs as hereditary determinant was first proposed by our laboratory in the case of paramutations, heritable gene-specific modulations of expression—a form of epigenetic non-Mendelian inheritance [19–21]. Expression of three genes, *Kit*, *Sox9* and *CDK9*, was observed to be increased after experimental transfer to the fertilized oocyte of sequence-related microRNAs. By the same approach, RNA-mediated non-Mendelian inheritance was subsequently reported in the case of induced neuropathological symptoms [22, 23]. RNA-induced paramutations were initially found to be transmitted for up to three generations in crosses with unaffected partners. Although such a short-term heredity may be significant when human diseases are considered, it does not appear to be a general feature of RNA transmission. We for instance observed that in serial brother-sister intercrosses between modified animals, the variant phenotype was maintained, apparently for undefined numbers of generations. This type of inherited long-term variation induced by homologous RNAs seems to be characteristic of a small number of genes that encode important pleiotropic regulators. In addition to *Kit*, *Cdk9* and *Sox9*, a number of other RNAs that we tested had no detectable effect when microinjected into single-cell embryos. We do not know yet why this observation applies to only one class of genes, and more investigations are needed. It may be significant that the genes that responded to homologous RNAs are haploinsufficient, indicating that the phenotype is sensitive to changes limited to one of the two alleles.

Founder animals raised on a high-fat diet show changes of their transcriptomes in all tissues, including modifications of the sperm microRNAs [14], which thus appeared as potential vectors that could initiate variation in gene-expression programs and transmission to future generations. On this basis, we hypothesized that sperm RNA acts as transgenerational signals of metabolic syndrome. Results in our laboratory [5] have indeed shown that microinjection of the sperm RNA of obese and diabetic males into naïve fertilized eggs leads to efficient transmission of the disease to the progeny. A possible level of regulation in several models of RNA-mediated heredity (reviewed by Chen et al. [13]) was initially suggested by our observation of a requirement for site-specific methylations of the inducer RNAs by the methyltransferase *Dnmt2* [24]. A negative mutation of the locus, while reducing the stability of several RNAs [25], abolishes induction of epigenetic variations.

Discussion

How sperm RNAs could be modified depending on the diet clearly requires further investigations. Transfer of RNAs from somatic to germ cells has been considered on the model of the small RNAs and tRNA fragments. ‘Epididymosomes’ (vesicles that fuse with sperm during epididymal transit) were considered as possible vehicles [26] but gene-specific regulation by such tiny RNAs is still to be demonstrated. To cite main contributors to the field [26],

‘that said, the hypothesis that sperm RNAs could be responsible for programming offspring phenotype presents several challenges for current mechanistic models for small RNA function. First, mammalian sperm carry extremely low levels of RNA and, considering the volume of a sperm relative to the oocyte, suggests that sperm are unlikely to carry enough RNAs to significantly alter the concentrations of a given RNA species in the oocyte, unless the RNA in question is absent or nearly so from the oocyte. This concern could be alleviated where sperm RNAs to be uniquely modified or pre-bound by an effector protein, but in any case, the

simplest model of sperm delivering a pool of soluble small RNAs must contend with the issue of the miniscule sperm cytoplasm'.

That being said, very recent results from our laboratory (M.R., A. Zarifi-Zarchi and F.C., unpublished) suggest a different model based on the finding of a mode of transfer by the sperm of non-coding RNA maintained in the spermatozoon not as soluble molecules but in the form of R-loop complexes with complementary genomic DNA. Recent studies by others also conclude to the possibility of the maintenance of RNAs bound to DNA in R-loop complexes [27–29]. Since the issues mentioned in the above reference concern only a 'pool of soluble RNAs' (the latter being anyway essentially removed in the residual body at the final stage of sperm maturation), they do not apply to RNAs engaged in R-loops. Such a model could obviously account for all the above objections but remains at this stage entirely speculative. Other possibilities certainly exist and are to be considered and investigated together with a number of other intriguing aspects. Among them is the nature of a general signal that modulates gene expression in all the cells of the body from somatic cells to the germ line. That is already plausible because on high-fat diet gene expression is highly changed not only in the liver but also in testes and sperm. We most probably must deal with a signal perceived by cells of the body including germ cells in response to a given RNA.

We are currently observing an exciting update to the nature vs. nurture paradigm, in which environmental effects alter genomic expression. The current development of experimental systems is leading to the accumulation of data that will lead to new hypotheses to explain other phenotypes and/or chronic non-genetic diseases. These developments also suggest that the knowledge of the vectors of inheritance will help in evaluating disease risk. Specifically, the identification of epigenetic markers in gametes (sperm) may predict disease susceptibility in offspring.

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