What the Mother Gives...*

Beena Pillai

Life starts from a single cell formed by the union of the sperm and egg. Genes inherited from the parents influence many features of the offspring – from physical appearance and disease susceptibility to more complex traits like behavior and cognitive abilities. Inherited RNA, both protein-coding and non-coding, transferred from the parents to the zygote can modify the information in the genome of the offspring during the early stages of development. Emerging evidence supports the possibility that non-coding regulatory RNA inherited from the egg and sperm may shape the offsprings' genome. The RNAs may mark positions in the genome that need to be activated at a precise time during development. They may also demarcate genomic landmarks like telomeres and centromeres. Differences in the type and amount of inherited RNA can result in inter-individual variability. By holding together distant regions of the genome, they may shape the 3D genome organization. These direct regulatory roles during the early stages of development may have long term consequences on the expression of genes and eventually on the traits of the offspring.

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In many cultures, the mother is exalted as the primary caregiver, thought to be in a privileged position to influence the future generation. The old saying "The hand that rocks the cradle rules the world" is indeed a reflection of this deep-seated notion. Octopus mothers undergo violent behavioral changes and starve themselves to death after caring for their large broods, although we do not know if this in any way benefits the offspring. In many animals, perhaps most visibly in humans, the mother spends considerable time actively teaching and demonstrating survival skills

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preparing the offspring for life ahead. In some species, the male may take over these responsibilities, as it happens in the sea horse. Besides these complex and apparently conscious efforts to improve the chances of survival of the next generation, there is a more deep-seated involuntary transfer of information from the parents to the next generation through genes. Many traits are inherited from parents through genes, almost universally encoded in the DNA of the organism.

Barring a few exceptions, eukaryotic genomes, spanning the bewildering array of organisms from yeast to humans are diploids i.e. they carry two copies of every gene, one each from the male and female parent. The DNA of the organism is thus a mix of possibilities thrown together at random from the parental genomes. The loss of a single copy of a gene or errors resulting in one bad copy may be masked by the presence of a good copy from the other parent. In other cases, both the copies called 'alleles' may work together to give an 'average' or combined output. Many complex multigenic traits and even common diseases may be the outcome of a large number of genes that get shuffled together in new combinations during the fusion of the gametes at the time of fertilization. The laws of inheritance of these traits through genes (made of DNA) arranged on chromosomes form the basic tenets of genetics. Yet time and again, empirical evidence has shown that there may be other extrachromosomal modes of inheritance.

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For instance, even in controlled lab studies, mice embryos seem to sense nutritional changes and modulate the expression of genes accordingly. In the real world, such an ability to modify the outcome of the inheritance through genes may indeed equip the embryo to respond to environmental fluctuations and make anticipatory adaptations. In humans and other viviparous animals, the developing embryo, held within the body of the mother is taking in nutrients and experiencing many changes through the mother's body signals. It is not surprising, therefore, that the embryo adapts to the changes its senses. But what may be the mechanism used by the embryo, and how exactly does its genome get tweaked in response to these signals without making irreversible

changes to the DNA itself? Before elaborating any further on the mechanisms of 'epigenetic inheritance'[1] and how it is distinct from the genetic mode of inheritance let us examine some of the most striking evidence in support of non-genetic or extrachromosomal inheritance.

[1] The study of heritable changes in gene function in the absence of a change in DNA sequence.

Researchers working with model organisms like mice can simulate this situation of non-genetic inheritance in the controlled conditions of the lab. 'Agouti' is a French-derived name for some large, lightly colored rodent species found in Central and South America. But amongst mice breeders and geneticists, the name refers to a mutant[2] that is strikingly obese and yellow in color. In animals from dogs to horses, breeders use this locus[3] to alter coat color. This variant can be tracked down to the second chromosome of mouse wherein the expression of a pigmentation gene is altered. Even though the DNA sequences remain identical, these superficial marks allow the gene to be suppressed in brown skinny mice. If the agouti mother is fed diet enriched in vitamin B12, choline and betaine the gene and the resulting yellow color are switched off in the offspring.

[2] An organism arising from a heritable change in the DNA.

[3] A region of the genome; a fixed position on the chromosome.

The agouti locus is perhaps the best studied case of nutritional changes switching off genes and altering the offspring phenotype in a dramatic way. Since then, often with gaps in our understanding of the molecular basis, several environmental factors have been linked to epigenetic changes. Maternal nutrition and stress have been known to trigger epigenetic changes during narrow windows of vulnerability in development, modulating traits like cocaine addiction and aggravated response to stress when the offspring grow into adults. A rodent mother takes care of the newborn pups by licking and grooming them. However, stressed mothers tend to spend less time in these activities. The pups that received such care from their biological mother or a foster mother tend to grow up into adults that respond to stress in a normal manner. However, pups that were deprived of such care display an exaggerated or inappropriately high response to stress as adults. Clearly, experiences of newborn pups were 'remembered' and perhaps programmed the brain to respond in predictable ways

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several months later.

This abiding memory is linked to the altered expression of genes, even though there is no permanent change in the DNA sequence. What is the chemical nature of these epigenetic changes that have a long-lasting impact on genes expressed in the offspring? The best studied modification of DNA bases is the 'methylation of cytosine'. Methylated cytosine in the regulatory regions of genes can switch them off. These marks are deposited by enzymes called 'methyltransferases' that accept methyl groups from metabolites providing a clear link between maternal diet and epigenetic marks. In the agouti mice, loss of methylation in the regulatory region of a pigmentation gene leads to the altered coat color. Maternal care induced changes in stress response are linked to the hypermethylation of DNA of the glucocorticoid receptor gene. Removing the hypermethylation marks through a variety of methods also reverses the aberrant stress response to an extent. Although the best understood, methylation of DNA is not the only mechanism by which such semi-permanent memory marks can be used to set the expression of genes (*Figure* 1). Genes are not only switched on or off but can be expressed at varying levels, much like a tunable light switch. A tunable switch can be operated through a completely modulatable knob, or it may snap to preset 'low', 'medium' or 'high' levels. The influence of epigenetic marks is analogous in that the expression level of a gene is firstly rendered permissible by the opening up of heterochromatin, the tightly wound coiled form of DNA associated with proteins called 'histones'. The open chromatin conducive for expression is composed of 'nucleosomes', DNA wound around the lysine-rich histone proteins. Transcription factors drive the expression of genes by displacing these nucleosomes and allowing the RNA polymerase to read through the DNA and create mRNA copies which in turn are translated into proteins by the cell. As mentioned before, the DNA itself maybe methylated modulating the access of transcription factors to gene regulatory regions. Besides the direct methylation of DNA, the histone proteins may be modified through methylation or acetylation of amino acid residues

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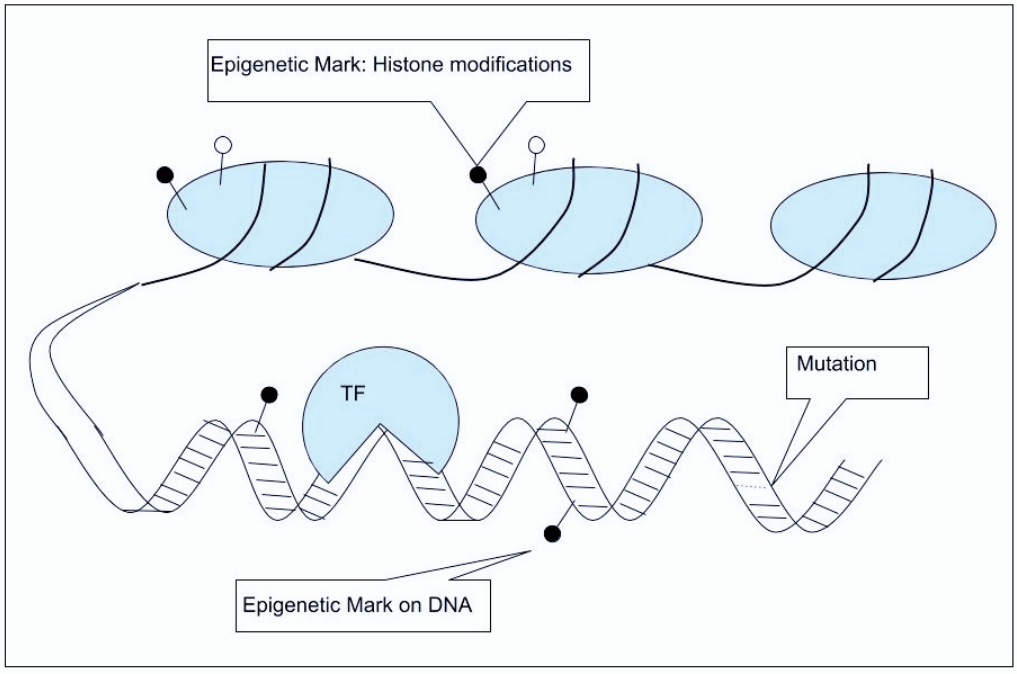

Figure 1. Chromatin and epigenetic marks: Epigenetic marks (black circles) are modifications made on DNA or histone proteins. DNA is wound around histone proteins to form nucleosomes (oval). The epigenetic marks modify accessibility of the DNA to transcription factors (TF) and set the gene expression level.

like lysine and arginine. DNA methylation is carried out by specialized enzymes called DNA methyltransferases while the histone modifications are deposited, read or erased (removed) by a large number of histone modifying enzymes. It is clear that these enzymes need additional factors that guide them to appropriate stretches of DNA sequence.

Amongst the macromolecules in the cell, proteins and RNA have the ability to interact with DNA in a sequence-specific manner. Proteins fold into complex 3D structures that recognize DNA through charge and shape contours making it difficult to have a one to one arrangement of DNA sequence and amino acid sequence engaged in DNA-protein interaction. However, intuitively, it appears that this complex shape-based interaction can accommodate a range of affinities making it suitable for one (protein) to many (DNA sites) interactions. These interactions maybe multivalency or multi-occupancy facilitating the coordinate regulation of genes by overcoming the problems imposed by the large distance between genes. On the other hand, RNA-DNA interac-

tions are fundamentally different since they are driven by the constraints of sequence-based interactions. Thus, protein-DNA interactions may be considered analogous to marbles strewn on the undulating surface of a crumpled blanket, running through burrows and ridges while RNA-DNA interactions maybe thought analogous to a zipper. An RNA molecule by virtue of its sequence has the best chances of finding a suitable DNA site for binding in its own site of origin. Besides this, it may also interact with other DNA regions with similarity, thus providing a range of affinity for its targets.

Indeed, the eukaryotic cell produces an amazing diversity of RNA molecules that regulate gene expression. In the human genome, for instance, there are barely 20,000 genes accounting for about 2% of the genome. However, the genome itself is pervasively transcribed producing RNA transcripts from nearly 80% of the genome. Of the total RNA produced in the cell, 95% or more comes from a single locus and forms the structural scaffold of ribosomes. Another 2–3% derived from a few hundred loci in the genome also form RNA molecules that are part of the machinery for gene expression. It would not be entirely wrong to say that 98% of the RNA produced in the cell comes from merely 1% of the 'genome real estate' and forms the 'infrastructure' required to manage the expression of the rest of the 2% RNA that arises from several 1000 genes. Majority of the transcripts, each expressed at very low levels, do not code for proteins. Instead, these RNAs modulate the expression of other genes. We refer to them as regulatory non-coding RNAs to distinguish them from the protein-coding mRNAs and the structural non-coding RNAs. The regulatory RNAs include the tiny 19–22 nucleotides long miRNAs, the slightly longer 40nts piRNAs and the larger than 200nts long non-coding RNAs (lncRNAs). All the three classes of regulatory non-coding RNAs can modify the epigenome of the cell.

Semi-permanent modifications deposited on the DNA or the histones that it is wound around can modulate the expression of nearby genes.

As mentioned before, semi-permanent modifications deposited on the DNA or the histones that it is wound around can modulate the expression of nearby genes. When DNA replicates prior to the birth of a daughter cell, the marks present on the DNA can

be read and transferred from the mother cell to the daughter cells. Thus a blood cell can put forth another blood cell which retains the entire genome but expresses only certain parts of the genome required for its identity. Here the mother to daughter transfer of fate decisions happens during the mitotic division of cells. In our body, such cell divisions are happening every minute, for replacing exhausted blood cells and worn out tissue besides during active growth. Epigenetic information is passed on from generation to generation through the gametes. The haploid genomes present in the gametes pre-fertilization is packaged into transcriptionally silent chromatin. Almost immediately after fertilization, the haploid genomes fuse together, and the zygote is formed. The newly formed diploid genome is transcriptionally silent during the first few cell divisions. The onset of transcription from the zygote varies in different organisms. In the fruit fly and zebrafish, it may take a few hours by when the embryo contains several cells. In the mammalian genomes, this event, also called maternal to zygotic transition, occurs as early as the two-cell stage, when the embryo is still in the fallopian tube making its way towards the uterus. The male pronucleus before its fusion with the female pronucleus undergoes active demethylation erasing the methylation marks from all but a few imprinted loci. Following this phase of active demethylation, over several rounds of cell division, the remaining methylation marks are also removed, and both the parental genomes are slowly cleared off their programming. It may appear then that the mother has no role to play in which of the zygote's genes will be activated. However, besides the genome, both gametes also package a whole contingent of proteins and RNA that direct the earliest events of the maternal-zygotic transition (*Figure* 2). This extrachromosomal inheritance is thought to be a way of passing on the basic infrastructure required to initiate the expression of genes from the zygotic genome; like the previous owner may leave the keys of the rooms for the benefit of the new owner during a house sale. Maternal inheritance refers to the transfer of traits from the mother through RNA and protein, and even mitochondria[4] deposited in the oocytes. The inheritance of maternal messenger RNAs is known to drive the establishment of

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[4]Sub-cellular organelles that carry their own DNA; mutations in the mitochondrial DNA can lead to inherited diseases.

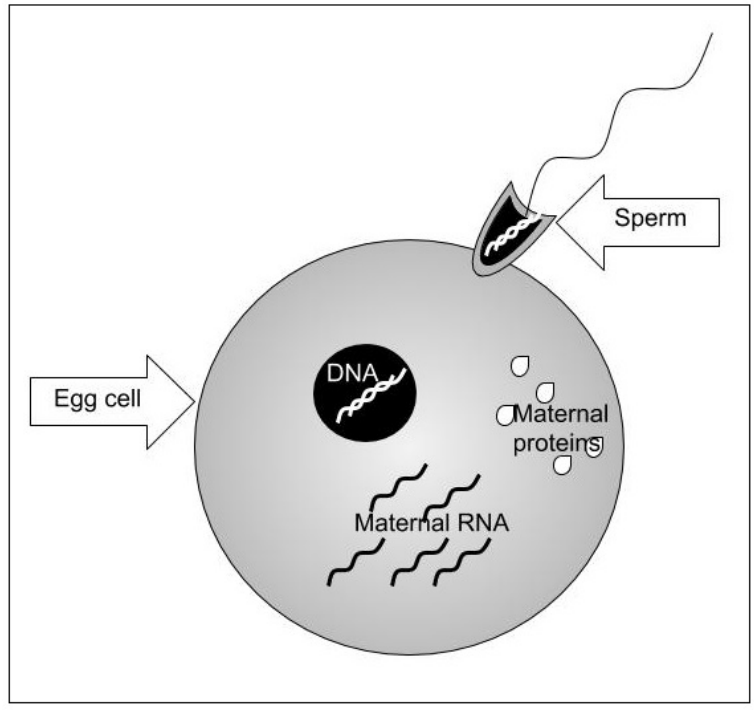

Figure 2. Fertilization is the process by which the male and the female gamete (sperm and egg cell) fuse to give rise to a zygote. Besides the DNA of both parents, the gametes also carry RNA, proteins and organelles that contribute to the information transfer from generation to generation.

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The regulatory non-coding RNAs present in the oocyte may influence the expression of genes from the zygote's genome, making this the third mechanism of epigenetic inheritance, in addition to DNA methylation and the post-translational modification of histone proteins. These mechanisms are not mutually exclusive. In fact, if these RNAs could direct the laying down of methylation marks in the zygotic genome, the expression levels of these genes could be scripted as per the information from the mother. Without permanent changes to the genome, zygotic gene expression could be tuned to low, medium or high levels (see above). The evidence in support of maternal, or even parental RNA as an epigenetic reprogrammer of the zygote is steadily mounting. As mentioned before, all classes of non-coding RNAs, miRNAs, piRNAs, and long non-coding RNAs can participate in the deposition of epi-

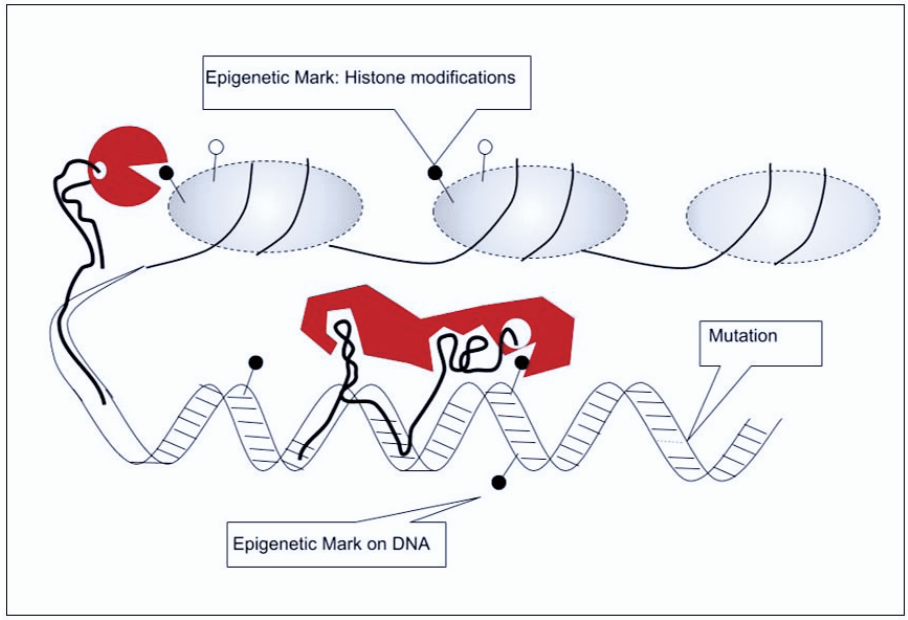

Figure 3. RNA can guide the deposition of epigenetic marks: Enzymes which can methylate DNA or deposit post-translational modifications on histone proteins may be guided to specific locations on the genome by long non-coding RNA which can recognize DNA regions with complementary sequences. The inherited maternal non-coding RNA can therefore guide zygotic genome activation.

genetic marks following the mitotic divisions of somatic cells or after fertilization of haploid gametes, thus affecting information transfer from cell-to-cell and generation-to-generation.

RNA molecules can direct the deposition of epigenetic marks on the genome. For instance, piRNAs can silence transposons by directing the deposition of repressive histone methylation marks on the transposon genes. Besides the demarcation of heterochromatin and euchromatin zones, maternally inherited non-coding RNAs could also potentially modulate the expression of specific zygotic genes (*Figure* 3). We found that a microRNA, miR-34 was inherited through the maternal cytoplasm in *Drosophila*. Reducing the expression of the maternally inherited miRNA even as the zygotic miR-34 was intact resulted in defects in the formation of the boundary between midbrain and hindbrain. Subsequently, other groups also showed that the level of miR-34 can influence the axes along which a cell separates into two during cell division. Although this was seen in stages when zygotic gene expression had started in earnest, it is possible that the orientation of cell division planes in the earliest divisions of the zygote are controlled

by the inherited miR-34.

In spite of looking carefully, only a few miRNAs have been shown to be inherited through the maternal cytoplasm of the oocyte. On the other hand, the search for long non-coding RNAs in the maternal cytoplasm paints a picture of a crowded milieu. Many long non-coding RNAs with known roles in chromatin modification are present in the cytoplasm of the oocyte, before zygotic gene expression is initiated. In organisms like zebrafish, where fertilization is external, and embryos transparent, it is, in fact, possible to manipulate the inherited RNA pool by specifically degrading a long non-coding RNA and monitoring changes during development. On the flip side, a single lncRNA can also be introduced into the single-celled embryo to study the impact it has on the zygotic genome. Introducing a single newly discovered lncRNA, *durga*, so named because it augments the expression of a nearby gene called *kalrn* (after Kali) into zebrafish embryos in the laboratory resulted in neurons with fewer dendrites, the branched outgrowths that help neurons connect with each other. Similarly, the reduction of a lncRNA called *cyrano* resulted in embryos with defects in brain morphology.

It is tempting to speculate that the differences in maternally inherited RNAs may indeed account for some of the inter-individual variability we see in the population. But to test this hypothesis, we would have to work with models where all the embryos have identical genomes but a variable pool of inherited RNA.

All RNA mediated epigenetic inheritance is not acquired from the mother. In recent years it has become clear that the sperm also carries its own contingent of non-coding RNAs of varying sizes. However, the overarching function of these RNAs and the consequences of some of these RNAs not being deposited by the parents into the gametes remains to be seen. It is tempting to speculate that the differences in maternally inherited RNAs may indeed account for some of the inter-individual variability we see in the population. But to test this hypothesis, we would have to work with models where all the embryos have identical genomes but a variable pool of inherited RNA.

Heritable changes in the genome over successive generations drives evolution. However, such genetic changes may not always be required by an organism, for example, the memory of rare, transient adverse environmental conditions should pass only to the immediate generation for better adaptation, and not to subsequent gen-

erations. In this regard, ncRNA inheritance is a tuneable form of heredity compared to rigid, long-term changes in the genome.

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Suggested Reading

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