Epigenetics and Developmental Plasticity

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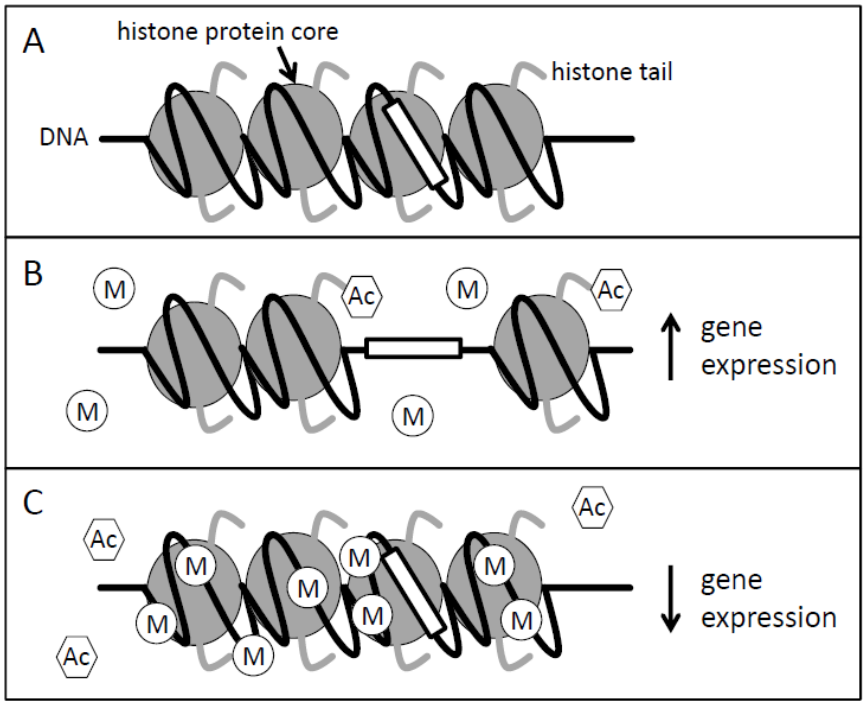
Development is a dynamic process, involving an elegant interplay between genes and the environment. Within an organism, this interplay leads to increasing cellular complexity and differentiation amongst genetically homogenous cells. In particular, epigenetic mechanisms, defined as factors that alter gene expression without altering underlying gene sequence, shape the activity of genes leading to the stable maintenance and heritability of cell-type specific patterns of gene expression. Thus, the transition from simple to complex is dependent on these gene regulatory mechanisms, which ultimately enable the generation of a diverse collection of cellular phenotypes from a single cellular genotype. A question of increasing theoretical and empirical interest is whether these same mechanisms can drive the emergence of phenotypic diversity at the level of the organism. In the context of discussions regarding the concept of ***developmental homology***, the notion of epigenetic mechanisms as a mechanism driving phenotypic diversity may be thought of as a highly conserved molecular pathway through which variation within and across generations may be achieved. To date, there are studies ranging from insects to humans that illustrate the role of epigenetic mechanisms in shaping divergent phenotypes. Moreover, epigenetic variation may be induced through particular environmental experiences and thus mediate the process of developmental plasticity. Here I will discuss several lines of evidence that, across species, epigenetic mechanisms have been implicated in the pathways through which the environment shapes the developing organism leading to stable variation in phenotype. Considered within an evolutionary perspective, it appears likely that these mechanisms are a critical feature of the process of adaptation to the environment, leading to adaptive reproductive, behavioral, and metabolic strategies which may enhance growth and survival.

Epigenetics & Development

Though development is certainly dependent on the "presence" of particular genes, as evident in the profound effect that gene mutations can have on mortality and morphology, the timing of gene activation and selective silencing of genes is equally critical. From a homogeneous cluster of progenitor cells, increasing cellular refinement and specialization is achieved through gene silencing – an outcome of epigenetic processes. Historically, the term ''epigenetic'' has been used to describe the dynamic interplay between genes and the environment which leads to variations in phenotype (Jablonka and Lamb, 2002). However, more current applications of this term are in reference to the specific molecular mechanisms which can lead to both transient and stable changes in the expression of genes. Gene transcription is dependent on the accessibility of DNA to RNA polymerase and other gene-specific transcription factors. Within the cell nucleus, DNA is wrapped around a core of histone proteins (see **Figure 1a**) which can undergo multiple post-translational modifications including methylation, acetylation, and ubiquination (Peterson and Laniel, 2004, Zhang and Reinberg, 2001). These modifications alter the dynamic interactions between the histones and DNA which either reduce or enhance the accessibility of DNA. In particular, histone acetylation (see **Figure 1b**) is associated with increased transcriptional activity whereas histone deacetylation or methylation is associated with transcriptional repression. The epigenetic process of DNA methylation represents what is generally considered a more stable and enduring modification to the activity of genes involving the conversion cytosine nucleotides to 5-methylcytosine. This process is mediated by methyltransferases which either promote maintenance (i.e. DNMT1) or de novo DNA methylation (i.e. DNMT3) (Feng et al., 2007, Razin, 1998, Turner, 2001). The conversion to 5-methylcytosine does not alter the DNA sequence but does reduce the likelihood that that sequence of DNA will be transcribed (see **Figure 1c**). The heritability of DNA methylation patterns is thought to be a critical feature of this epigenetic mark which allows cell-type specific gene expression patterns to be sustained during

mitosis. Gene deletion of enzymes involved in DNA methylation and histone acetylation leads to embryonic lethality – highlighting the importance of these epigenetic processes in development.

FIGURE 1. *Epigenetic regulation of gene expression*. A) Within the cell nucleus, DNA (black line) is coiled around a core of histone proteins (grey circles). The structure of these proteins includes a histone tail which dynamically interacts with DNA. Within the sequence of DNA, there are regions which are critical for the regulation of gene activity such as gene promoter regions (white rectangle). Access to gene promoter, is critical for gene expression. B) When DNA is unmethylated and histone tails are acetylated (Ac), there is increased accessibility to the gene promoter region, leading to increased gene expression. C) When DNA is methylated (M) and histones are deacetylated, gene expression is typically reduced.



Epigenetics, Environments, and the Origins of Phenotypic Diversity

The divergence in cellular phenotype than can be achieved through epigenetic pathways has lead to increasing speculation that divergence in phenotype of the individual (e.g. neurodevelopment, disease risk, behavior) can likewise be achieved though mechanisms such as DNA methylation. In monozygotic twins, there is emerging evidence for discordance in DNA methylation patterns and it would appear that this discordance increases over time (Fraga et al., 2005, Mill et al., 2006, Wong et al., 2010). The critical question raised by these findings is whether epigenetic modifications can be shaped by particular environmental experiences and whether these effects can contribute to our understanding of the long-term impact of these experiences. Across species, there is evidence for the influence of early life nutrition, stress, social experiences, and toxin exposures on epigenetic patterns that persist across the lifespan.

Epigenetics & Nutrition. Perinatal nutrition is a critical signal of environmental quality which can predict growth and survival of offspring. In human epidemiological studies, analysis of blood samples from famine exposed vs. non-exposed siblings indicates that there is decreased DNA methylation of the Igf2 gene as a consequence of maternal periconceptual exposure to famine (Heijmans et al., 2008). Laboratory studies in rodents have subsequently identified specific nutritional deficits, such as prenatal protein restriction or folic acid/choline deficiency as having similar epigenetic consequences. Offspring of female rats placed on a protein deficient diet throughout gestation were found to have elevated hepatic glucocorticoid receptor (GR) and peroxisomal proliferator-activated receptor (PPAR) gene expression associated with decreased DNA methylation of these genes (Lillycrop et al., 2005, Lillycrop et al., 2008). Epigenetic modifications in response to the nutritional environment during the early stages of development may also have implications for the morphological

changes associated with caste phenotypes in eusocial insects. Honeybees have functional DNA methyltransferases and the degree of methylation of the genome varies during the course of development (Wang et al., 2006). Amongst female honeybees, social/reproductive caste is determined through early nutritional exposure to royal jelly (with increased royal jelly promoting the development of queen bees and reduced royal jelly promoting the development of worker females – see **Figure 2a**). Manipulation of the activity of the DNA methyltransferase DNMT3 in honey bee provides evidence that DNA methylation mediates these divergent phenotypes. Under control conditions, 75% of larvae develop as worker bees whereas inhibiting DNMT3 leads to the majority of larvae developing morphologically as queen bees (Kucharski et al., 2008). Taken together, these studies illustrate how epigenetic mechanisms serve a central and developmental role across species, leading to individual variation.

Epigenetic Impact of Parental Care. Variation in postnatal maternal behavior may also induce epigenetic changes in offspring development. Postnatal maternal licking/grooming (LG) behavior in rats has been found to induce long-term changes in neuroendocrine function and behavior of offspring, with consequences for stress responsivity and cognition, and cross-fostering studies have confirmed that these effects are mediated by the level of maternal care received during postnatal development (Meaney, 2001). Analysis of the GR promoter region suggests that variations in GR expression associated with differential levels of maternal care are maintained though altered DNA methylation (Weaver et al., 2004). Thus, offspring who receive high levels of maternal LG during the early postnatal period have decreased hippocampal GR promoter DNA methylation, increased GR expression and decreased stress responsivity. In contrast, low levels of LG are associated with increased GR DNA methylation, decreased GR expression, and an increased hypothalamic-pituitary-adrenal (HPA) response to stress. Time course analysis has indicated that these maternally-induced epigenetic profiles emerge during the postnatal period and are sustained into adulthood. In humans, elevated GR methylation is found in fetal cord blood, associated with increased maternal depression, and this epigenetic modification predicts stress responsivity in infants (Oberlander et al., 2008). In postmortem brain tissue, a history of childhood abuse predicts elevated GR methylation and decreased hippocampal GR gene expression (McGowan et al., 2009). Similar to the case of early life nutrition, epigenetic mechanisms appear to play a significant role in linking the quality of parental care to long-term variation in offspring phenotype – particularly phenotypes related to stress responsivity.

Adaptive Function of Phenotypic Variation

The ability to change in response to environmental cues has typically been considered an adaptive strategy, allowing an organism to shift phenotype towards that which is most suited to the current environmental conditions. It will often be the case that the quality of the environment experienced during early development will be very similar to that which will be experienced in adulthood. Thus, epigenetic mechanisms which enable stable changes to gene expression may allow for the stability of adaptations. It is often tempting to consider emergent phenotypes to be “good” or “bad” (as is often the case when discussing the variation in stress response associated with low vs. high maternal care). However, when we consider some of the examples of phenotypic plasticity that have been described so far, it becomes clear that a “good vs. bad” distinction is far too simplistic. The phenomenon of nutritionally induced caste differences in honeybees provides a route through which the social structure of the hive is maintained. Both queen bees and worker bees serve a critical role in the growth and survival of the hive – queen’s reproduce, workers nurture. The same logic can be applied to the effects of low vs. high levels of postnatal LG observed in laboratory rats. The outcomes of Low LG, such as heightened stress responsivity and reduced cognitive ability would certainly be consequences for offspring that are considered “not-optimal”. However, it is important to note that natural variations in maternal LG do not lead to increased mortality. Offspring of Low LG and High LG dams are equivalent on gross measures of health and welfare. Interestingly, despite

performing more poorly on cognitive measures under "standard" testing conditions, when offspring of Low LG dams are in a heighted physiological state of stress, they exhibit enhanced learning and hippocampal plasticity (Champagne et al., 2008). Thus, determining whether a rearing environment is "good" may be dependent on the quality of the environment experienced later in life. Low LG experience may be beneficial to the functioning of offspring living in stressful environments (e.g. enhancing stress-induced cognition or reacting to threats more rapidly due to enhanced HPA activity) whereas High LG leads to adaptive functioning in low-stress environments (e.g. increasing exploration leading to increased access to potential resources). Related to the idea of context-dependent benefits of high or low LG is evidence for trade-offs in reproduction that are observed in the female offspring of High LG and Low LG dams. Female offspring of Low LG dams exhibit reduced maternal care associated with epigenetic silencing of estrogen receptors in brain regions critical for maternal responsivity (Champagne et al., 2003, Champagne et al., 2006). However, despite this apparent reproductive disadvantage, there is emerging evidence that these females have heightened sexual receptivity and produce more litters than female offspring of High LG dams (Cameron et al., 2008). This enhancement in sexual behavior amongst offspring of Low LG dams is accompanied by up-regulation of the neuroendocrine systems which are associated with this aspect of reproduction. These findings suggest that reproductive strategies are shaped by mother-infant interactions and that though decreases in one aspect of reproduction may occur, there are compensatory increases in other aspects of reproduction that allow offspring to successfully reproduce. Similar to queen's and workers, offspring of low LG dams reproduce, while offspring of high LG dams nurture (see **Figure 2b**).

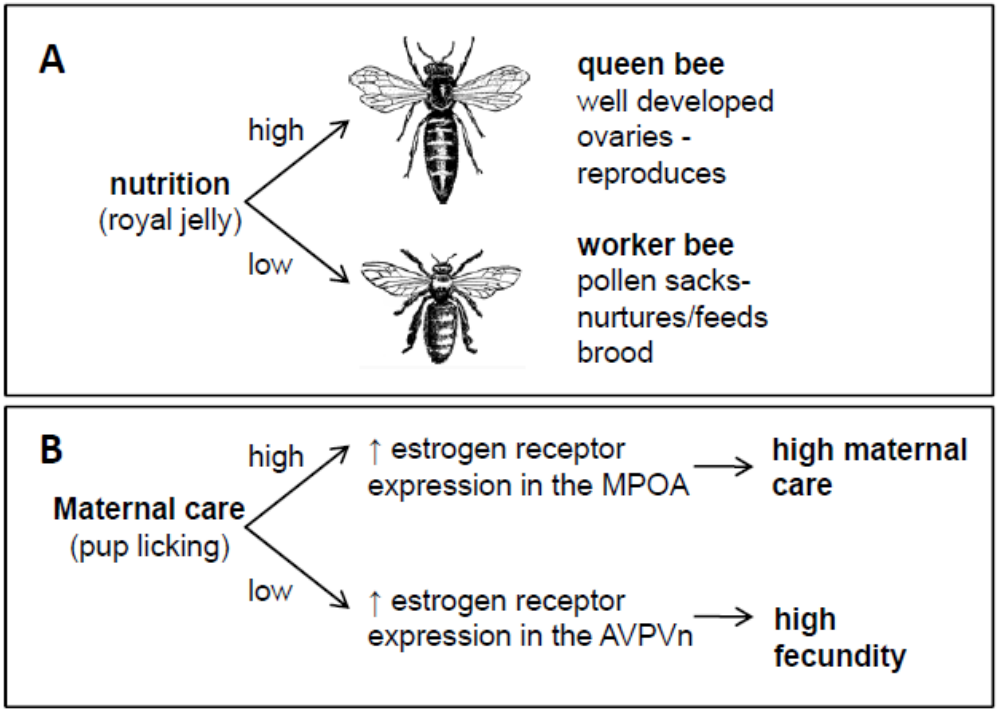


FIGURE 2. *Adaptations to early environmental signals leading to altered reproductive strategies*
A) Amongst honeybees, variation in the amount of royal jelly experienced in early development leads to phenotypic variation giving rise to the queen (the sole reproducers in the hive) or workers (females that provide care for the developing brood). B) In rats, high maternal care experienced in infancy leads to increased estrogen receptor gene expression in the medial preoptic area (MPOA) – a brain region critical for maternal care, whereas low maternal care is associated with increased estrogen receptor gene expression in the anteroventral paraventricular nucleus of the hypothalamus (AVPVn) – a brain region associated with sexual behavior/hormonal cycles.

Conclusion

The study of epigenetics is in its infancy and there are many gaps in our knowledge about the mechanistic link between environmental experiences and these molecular pathways. However, it is tempting to speculate that the mechanisms that evolved to maintain cellular differentiation can likewise shape phenotypic variation at the level of the individual. Across species, these mechanisms appear to play a critical role in the ability to adapt and "fine-tune" an organism's biology and behavior to meet the demands of the environment. Are these adaptations, like cellular phenotypes, heritable? This question has lead to a revival of Lamarckian theories which will certainly challenge and intrigue researchers in this field.

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