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The basis of bee-ing different: the role of gene silencing in plasticity

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Developmental plasticity can generate, from one genotype, diverse alternative phenotypes appropriate to local environmental conditions (West-Eberhard 2003). However, our understanding of the developmental-genetic mechanisms that underlie plastic responses remains incomplete. Recent research suggests that DNA methylation, a system of gene silencing heritable across cell divisions, may serve as a mechanism underlying the evolution of plasticity. In particular, several recent studies in Hymenoptera (ants, bees, wasps, and sawflies) highlight the potential importance of methylation for understanding plasticity (Wang et al. 2006; Kronforst et al. 2008; Kucharski et al. 2008).

DNA METHLYATION: AN OVERVIEW

DNA methylation is one of the most well-characterized epigenetic mechanisms. It involves the addition of a methyl group, typically to the number 5 carbon of a cytosine–pyrimidine ring that occurs right next to a guanine nucleotide, a so-called CpG site. Methylation of CpG dinucleotides reduces gene expression either by directly interfering with the binding of transcription factors or by facilitating the binding of methyl-CpG-binding proteins, which in turn initiate a cascade of events that ultimately results in the formation of compact and silent chromatin (Bird 2002). This silencing effect is inherited across rounds of cell divisions and sometimes generations, while the underlying DNA sequence remains unaltered. As such, DNA methylation provides a powerful and essential epigenetic mechanism for the regulation of development from basic cell differentiation to tumor formation (Hendrich and Tweedie 2003).

Several nonexclusive hypotheses aim to explain the evolution of methylation. DNA methylation may have evolved as a mechanism to silence transposable elements, regulate tissue-specific development, or reduce transcriptional noise (Walsh and Bestor 1999; Bird 2002). In mammals and flowering plants, intragenomic conflict and genetic imprinting provide another context that may have shaped the evolution of DNA methylation. Here, DNA methylation results in expression differences between maternal and paternal genomes, as in the maternal silencing (or paternal inheritance) of genes involved in rapid embryonic growth (Moore and Haig 1991; Reik and Walter 2001). Recent studies now provide strong support that methylation may also have evolved to function as a mechanism underlying adaptive phenotypic plasticity, and may have played an important role in the evolutionary diversification of plasticity.

TAXONOMIC DISTRIBUTION OF METHYLATION

Not only is DNA methylation able to regulate a wide range of developmental and cellular processes, but it does so in diverse eukaryotes, including fungi, plants, and vertebrates (Binz et al. 1998; Finnegan et al. 1998; Hendrich and Tweedie 2003). Insects, on the other hand, seemed like the odd one out: although evidence for CpG methylation exists from several insect species (Field et al. 2004), no DNA methyltransferases (DNMTs), the very proteins that carry out methylation (Jaenisch and Bird 2003), had been described. The only exception was a single and rather unusual DNMT in *Drosophila* and *Anopheles* (Marhold et al. 2004), dDNMT2, whose functional significance remains enigmatic. We would have expected to see at least two different DNMTs on the basis of what is known of other animals: one for de novo methylation (DNMT3) and one for maintenance methylation (DNMT1).

Recent work on hymenopterans in general and honeybees in particular has now clearly demonstrated that DNA methylation appears to function in insects in a way similar to that in mammals. Wang et al. (2006) showed that the honeybee *Apis mellifera* has a fully functional CpG methylation system complete with both DNMT3 and not just one but two DNMT1s, DNMT1a and DNMT1b. The same study also provided the first compelling evidence that DNMT activity differs depending on sex, developmental stage, and tissue, suggesting that DNA methylation in *Apis*, and perhaps insects in general, exhibits the same complexities as has been observed in other phyla. But important differences also began to emerge. Firstly, DNA methylation in vertebrates functions to a significant degree in the repression of repetitive DNAs and retrotransposons, presumably to maintain genome integrity

(Bird 2002); yet, the same genome elements appear not to be methylated in *Apis*. Instead, methylation appeared limited to coding regions. Secondly, *Apis* emerged as the only taxon thus far with two paralogs for the maintenance of methylation. These details aside, however, the findings clearly demonstrated the existence of a complete and functional methylation system in an insect. And that was only the beginning . . .

A recent study suggests that DNA methylation is not restricted to honeybees but instead is widespread and diverse across the Hymenoptera. Kronforst et al. (2008) digested genomic DNA of three adult individuals of 12 species of wasps, bees, and ants with two restriction enzymes that targeted the same cut site (5′-CCGG-3′) but exhibited different sensitivities to methylation, and were thus able to get a basic estimate of the proportion of restriction sites that were methylated in each individual. Out of a mean of 580 restriction sites across taxa, the proportion of methylated sites ranged from 1% to 19% depending on species. The authors used the same approach to contrast developmental stages in *A. mellifera* and found that the proportion of methylated restriction sites dropped from 11% during larval and early pupal development to 4.6% in adults. Furthermore, some sites varied in the incidence and degree of methylation between individuals. These data illustrate that CpG methylation is common yet variable across social Hymenoptera as well as during development. This recent work in Hymenoptera has now opened the door for studying the functional implications of methylation in insects, including its potential role in developmental plasticity.

METHYLATION: A POTENTIAL ADAPTIVE RESPONSE TO ENVIRONMENTAL STIMULI

Methylation is often responsive to environmental input, but recent work in social insects has extended the implications of this observation from a disease context (e.g., Jaenisch and Bird 2003; Feinberg 2007) to that of developmental plasticity. Honeybees are known to respond to nutritional cues during larval development by developing into reproductive queens or sterile worker morphs. Kucharski et al. (2008) inhibited methylation during *Apis* development and found that variation in methylation patterns produced alternative phenotypes comparable to those resulting from nutritional cues. Specifically, they used RNA interference to knockdown *Apis*-DNMT3, the transferase responsible for de novo methylation, in newly hatched L1 larvae; qPCR and in situ hybridization revealed that silencing was strongest during the larval period when nutritional cues trigger worker or queen development. Among control-injected animals, approximately 77% emerged as workers (with 2–6 ovarioles/ovary) and 23% as queen-like individuals (with 50–80 ovarioles/ovary). Among DNMT3-RNAi individuals, the authors found 28% workers with rudimentary ovaries and 72% queens with fully developed ovaries (120–190 ovaries/ovary). It seemed that silencing DNMT3 prevented worker-destined larvae from silencing ovarian maturation and caused a much larger proportion of individuals to emerge as fully reproductive queens, rather than nonreproductive workers.

The authors then examined a particular gene, *dynactin p62*, which has been shown to be (a) more methylated in worker than queen *Apis* and (b) responsive to nutritional cues in *Drosophila*, suggesting a possible role in linking environmental input to methylation and plasticity. They showed that DNMT3-RNAi was able to reduce *dynactin p62* methylation to a level very similar to what was normally observed in presumptive hive-reared queens but not workers. Most strikingly, when the authors examined exons 5–7 of *dynactin p62*, they found that the methylation status of CpG sites in future hive-reared versus DNMT3-RNAi-induced queens exhibited remarkable similarities. Moreover, active methylation (and/or demethylation) appeared restricted to tissues undergoing active DNA replication, such as the *corpora allata*, a gland that plays critical roles in hormone (JH) release and caste determination (Nijhout 1994). Finally, microarray experiments then showed that DNMT3-RNAi resulted in the altered expression of a battery of genes, including many relevant to metabolism, growth, and endocrine regulation. Importantly, overall changes in gene expression were similar between DNMT-3RNAi individuals and hive-reared queen-destined larvae. In particular, genes responsive to nutritional inputs (e.g., one in the TOR signaling cascade) showed differential expression in DNMT3-silenced and control individuals. Overall, methylation patterns, gene expression, and reproductive phenotypes are mirrored in diet (royal jelly) and DNMT3-manipulated individuals, suggesting that nutritional cues that regulate plasticity in bees may be underlain by changes in methylation patterns.

A GENERAL ROLE OF METHYLATION IN PHENOTYPIC PLASTICITY AND DIVERSITY?

The results summarized above provide the first evidence that DNA methylation mediates the regulation of reproductive and nonreproductive castes in honeybees (Kucharski et al. 2008), that methylation effects parallel those of nutritional input in caste determination (Kucharski et al. 2008), and that methylation is widespread yet diverse across the Hymenoptera (Kronforst et al. 2008). These findings now elevate DNA methylation from a potential mediator of intragenomic conflict in Hymenoptera to a major regulator of the environment-dependent expression of complex traits in general. This also raises the possibility that evolutionary changes in the degree to which target genes are methylated, changes in the identity of target genes, and changes in the degree to which methylation is sensitive to descent, nutrition, or some other factor

may be providing previously underappreciated opportunities for rapid phenotypic diversification, including the diversification of plasticity.

These studies highlight a growing interest in how methylation may serve as a mechanism underlying phenotypic plasticity. Reprogramming of cells through methylation following environmental input would provide an efficient mechanism of adapting gene expression in later development to local conditions. Studies of developmental plasticity are now primed to test for the prevalence and importance of methylation in both animals and plants (Bruce et al. 2007). The door has also been opened to consider the role of methylation in crossgenerational plasticity (e.g., maternal effects) and whether alternate mechanisms of transcriptional memory (e.g., polycomb–trithorax complex, Francis and Kingston 2001) are also relevant to developmental plasticity.

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