Epigenetic switch turns on genetic behavioral variations

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What roles do environmental factors, genes, and heredity play in shaping the behaviors of individuals? In his 1963 article entitled "Behavior genetics and individuality understood," the pioneering behavioral geneticist Jerry Hirsch (1) eloquently articulated that "Individual differences are no accident. They are generated by properties of organisms as fundamental to behavioral science and biology as thermodynamic properties are to physical science." In the era of molecular genetics and genomics, understanding neuronal activity and organismal behavior at the cellular and molecular levels has rapidly progressed, primarily via studies of behavior in genetically tractable organisms, such as the fruit fly, the worm, the laboratory mouse, and others. Nonetheless, over 50 y after Hirsch's seminal article, and despite the aforementioned scientific advances in cellular and molecular neurobiology, understanding the specific contributions of natural genetic variations to phenotypic diversity between individuals and across populations remains a major research challenge. The reasons for why identifying the actual genetic elements that underlie natural behavioral variations between individuals is difficult are deeply embedded in the polygenic nature of the majority of complex phenotypes, including behavior (2, 3), and the often complex, nonlinear relationship between plastic primary gene products, RNAs and proteins, and the organismal phenotypes they influence. Now in PNAS, Anreiter et al. (4) elegantly demonstrate that the previously identified (5), naturally occurring allele-specific effects of *foraging* (*for*) on individual behavioral foraging decisions of larval and adult *Drosophila* depends on the epigenetic action of the *dG9a* gene (4).

The PKG-Dependent Signaling Pathway Is a Conserved Modulator of Feeding and Food-Search Behaviors

The polymorphic *for* locus, first discovered by Marla Sokolowski when she was still an undergraduate student (6), represents a classic example of how natural genetic polymorphism in a single major gene can have a significant effect on ecologically relevant behavioral variations between individuals across a single population (5, 7, 8). Initial observations indicated that natural wild-type populations of *Drosophila melanogaster*, at both the larval and adult stages, exhibit variable foraging strategies when food is abundant (9). Later, it was shown that individual foraging strategies are surprisingly influenced by natural genetic variations in a single major locus. Careful genetic analyses indicated that two major alleles of *for*, termed *sitter* and *rover* (for^{s} *and* for^{R}), are

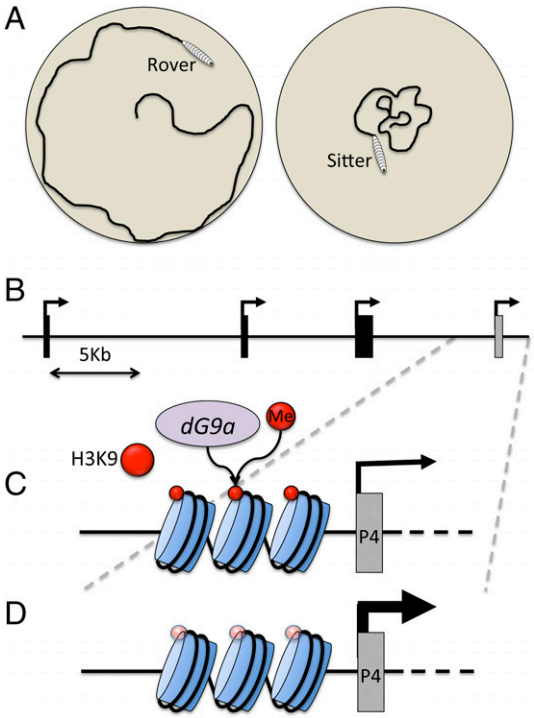

Fig. 1. Natural differences between individual *Drosophila* food search behaviors are affected by allelic variants of the *foraging* gene. (*A*) When on nutritive yeast substrate, rover larvae foraging trails are significantly longer than those of sitters (4). (*B*) The *for* genomic locus includes four alternative transcriptional start sites (P1–P4). Boxes represent alternative first exons. (*C*) In rovers, an interaction with the histone methyltransferase *dG9a* (28) leads to a higher methylation of histone 3 at the lysine 9 position (H3K9), and therefore, lower *for*-P4 transcriptional activity relative to sitters (*D*).

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maintained in wild-type populations by density-dependent selection (8, 10, 11). As their names suggest, when food is abundant homozygous "sitter" larvae forage over small areas while homozygous "rover" flies tend to cover a significantly larger area (Fig. 1*A*). Once cloned, *for* was shown to encode a cGMP-dependent protein kinase (PKG), which exhibits higher mRNA expression and enzyme activity levels in homozygous *rovers* relative to homozygous *sitters* (5).

How quantitative variations in PKG activity might affect the activity of specific downstream genes in the context of feeding and foraging behaviors remains mostly unknown. Nevertheless, the role of *for* and PKG signaling in the regulation of behavioral plasticity in general, and insect foraging in particular, seems to be evolutionarily conserved. For example, studies in the eusocial honey bee, *Apis mellifera*, have demonstrated that the expression of *Amfor* and PKG activity increase in the brains of worker bees as they make the transition from in-hive tasks to foraging outside the hive (12–14), and treatment of young bees with a PKG activator results in a precocious transition to foraging activity, possibly by switching the innate phototaxis behavior of workers from negative to positive (13, 15). Following the studies in the fly and honey bee, *for* and cGMP signaling were further implicated in the regulation of feeding and food-search behavior in diverse insect species, including studies in various ant species (16–18), bumblebees (19, 20), social wasps (21), beetles (22), locust (23), as well as in nematodes and other animal clades (24–26).

Epigenetics, Allelic Variations, and Individual Behaviors

Although the simplest model for phenotypic differences between conspecifics is often assumed to be genetic, when it comes to plastic phenotypes, such as behavior, the associations between specific haplotypes and observed individual phenotypic values are often blurred, and therefore difficult to interpret at the molecular and cellular levels. Therefore, the original reports about a clear association between specific alleles of *for* and individual differences in food search behaviors in *Drosophila* became an instant classic example of how a major gene might affect natural behavioral differences. However, although the allele-specific contribution of *for* to the *Rover–sitter* behavioral polymorphism was established genetically, the actual molecular mechanism that leads to the phenotypic variation eluded investigation.

Initially, the cloning of *for* and subsequent analyses of the relationship between behavioral phenotypes and PKG activity suggested that the rover phenotype is a product of higher *for* expression levels, and PKG activity in the nervous system indicated that the primary allelic-dependent phenotypic difference between for^R and for^s are quantitative rather than qualitative structural variations in the protein encoded by the locus (5, 27). These findings suggest that the elusive molecular differences between the two *for* alleles are buried in the regulation of its expression via one of its four transcriptional start sites (Fig. 1*B*). One possible molecular mechanism that could explain the differential expression levels of *for* between sitters and rovers could be by stable chromatin modifications of the locus by epigenetic factors. Indeed, transcriptional analyses revealed that activity of one of *for*'s promoters was higher in rovers relative to sitters via allele-specific interaction of *for* with the epigenetic factor *dG9a*, a histone-lysine *N*-methyltransferase (4). However, in contrast to the initial findings, promoter-specific analyses of *for* expression revealed a *dG9a*-depedent suppression of P4 promoter-specific expression in rovers relative to sitters (Fig. 1*C*), which explains the majority of the observed phenotypic difference between the *rover* and *sitter* adult genotypes.

Implications and Future Directions

Despite the vast advancements made in understanding biology in molecular terms, understanding what role genetically encoded information plays in shaping the behavior of individuals remains mostly a mystery. Highlighted by the 2017 Nobel prize in Physiology or Medicine, which was awarded for the discovery of the molecular mechanism that keeps time in cells, studies of genetically tractable organisms, such as *Drosophila*, will continue to be essential for deciphering the rules, codes, and principles that govern organismal life at the cellular and molecular levels. Along these lines, the findings by Anreiter et al. (4) are exciting for several different reasons. First, they now provide a possible molecular explanation for the elusive *rover–sitter* differential allelic effect on behavior. Second, they help explain how relatively simple binary allelic combinations of a single major gene could still generate quantitative, rather than simpler Mendelian, phenotypic distributions across individuals in a population. Third, because the role of *for* in regulating feeding and food search behaviors is conserved across different animal species, these findings suggest that epigenetic factors, such as *G9a*, may play a role not only in driving long-term stable allelic differences, but also in allele-independent spatial and temporal regulation of *for* expression at the physiological timescale, and therefore, in behavioral plasticity exhibited by individuals. Finally, they provide conceptual and empirical frameworks for understanding how the interplay between environmental factors, epigenetic regulation of gene action, and genotypes define and refine individual behavioral outcomes.

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