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Genes for Normal Behavioral Variation: Recent Clues from Flies and Worms

Minireview

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The question of how genes contribute to normal individual differences in behavior has captured our imagination for more than a century. Several fundamental questions come to mind. How do genes and their proteins act in the nervous system and in response to the environment in order to cause individual differences in behavior? Do genetic differences between natural variants arise from alterations in the structural or regulatory regions of a gene? Can we predict which genes for behavior, identified by mutant analysis in the laboratory, will have natural allelic variation? Three groundbreaking studies (Osborne et al., 1997; Sawyer et al., 1997; de Bono and Bargmann, 1998) published in the past year demonstrate that we now have the knowledge and technological capability to address these questions empirically. Each study has successfully identified a single major gene for a given behavior and, with the aid of transgenic animals, shown that its gene product is responsible for naturally occurring individual differences in that behavior.

The experimental strategies used in these studies can be classified according to how the research evolved. In one case, variation in the protein of a previously cloned gene was identified, and later naturally occurring behavioral variations that resulted from differences in the protein were found (the number of threonine–glycine (Thr–Gly) repeats in the *period* (*per*) gene of *Drosophila* [Sawyer et al., 1997]). In the other case, natural behavior variants were discovered, and a single gene responsible for these behavioral differences was localized and cloned (e.g., rover and sitter larval foraging behavior in *Drosophila* [Osborne et al., 1997] and social and solitary foraging behavior in *C. elegans* [de Bono and Bargman, 1998]). The first approach proceeds "from gene to protein to natural variant" and the second one "from natural variants to gene to protein." A third approach that has the potential to identify natural genetic variants with relatively small effects on behavioral phenotypes is quantitative trait locus (QTL) mapping, a technique that uses densely distributed molecular markers in recombination mapping in order to determine which loci segregate with natural variation for a given phenotype.

It comes as no surprise that only a smattering of naturally varying behavioral genes are known since genetic dissection of behavior has been based on the analysis of mutants. The study of single gene behavioral mutants, the approach pioneered by Benzer and colleagues, has been invaluable for shedding light on the molecular and neural bases of behaviors such as learning, courtship, and circadian rhythms (reviewed by Tully, 1996). This genetic dissection approach assumes that there is only one "wild-type" or "normal" phenotype and that mutants alter this wild-type form. Undeniably most, if not all, behavioral mutants generated in the lab would not survive in nature. Consequently, this approach has told us little about the genes involved in naturally occurring individual differences in behavior. This is in contrast to the older school of *Drosophila* behavior genetics (founded by J. Hirsh in the 1950s), which artificially selected for behavioral differences using wild caught flies (see Tully, 1996). These behavioral geneticists were interested in why individual differences in behavior had evolved and how variation is maintained in natural populations. Most often, they found that many genes (polygenes) contributed to the behavioral differences between the artificially selected strains. It was assumed that each polygene had small, cumulative effects on the behavioral phenotypes and that they were therefore unmappable. The three papers that will be discussed in this minireview prove that major gene effects are responsible for some natural variation in behavior and that the molecular basis of these genes can be understood. They demonstrate that studies of natural variants do provide insight into both the mechanistic and evolutionary significance of normal variation in behavior.

In Sawyer et al. (1997), natural variation in a behavioral phenotype was shown to result from a molecular polymorphism. Specifically, the temperature compensation ability of the circadian clock was shown to be due to a polymorphism within the *per* gene. The *per* gene in *Drosophila* was originally identified in a mutagenesis screen for eclosion rhythms and was later shown to affect a number of rhythms in the fly, including circadian locomotor activity rhythms and courtship love song ultradian rhythms. Molecular analysis of *per* and another clock gene in *Drosophila* called *timeless* showed that both proteins are involved in an autoregulatory feedback loop critical for circadian rhythmicity. The *per* gene has a repetitive region which has a Thr–Gly encoding repeat that is polymorphic in length. Two of the major variants, (Thr–Gly)17 and (Thr–Gly)20, are found along a north–south cline in Europe. These variable repeats appear to be related to how well a fly can maintain its circadian rhythm as temperature varies, a property termed temperature compensation (Hall, 1997). Transgenic strains were used to investigate whether the number of repeats (17 or 20) affected the temperature compensation ability of the clock at 18°C and 29°C. Sawyer et al. (1997) transformed embryos carrying null alleles of *per* (per^{01}) with either 0, 1, 17, or 20 Thr–Gly repeats and argued that the 20 Thr–Gly repeat transformants showed better temperature compensation than did the transgenic flies with 17 Thr–Gly repeats. The uniqueness of this study lies in the fact that they used a gene, originally identified in the lab, to investigate the evolutionary significance of molecular variation in that gene in nature. Their results suggest that other genes identified in the lab by mutagenesis could be used to study the mechanistic and evolutionary basis of natural variation. The difficulty in this approach is that the genes to choose are at this point anyone's guess, because we just don't know enough about how to identify genes important to natural behavioral variation.

Other studies have used a different approach to identify genes involved in behavioral variation. Osborne et al. (1997) investigated the molecular genetic basis of natural variation in fruit fly foraging behavior. Fly larvae forage for food (yeast and water paste) in one of two ways: as a rover or a sitter. As their names imply, rovers exhibit longer foraging paths than do sitters (reviewed by Partridge and Sgro, 1998). Rover larvae also move between patches of food, whereas sitters tend to remain feeding within a food patch. Rover paths are straighter than those of the sitter, which exhibit higher turning rates on food. In the absence of food, both variants move at similar rapid speeds, indicating that sitters are not simply sluggish animals. A combination of quantitative genetic and Mendelian genetic analyses showed that the rover/sitter variants resulted from a single major gene in *D. melanogaster*, which we called *foraging* (*for*). In larvae, the rover allele (for^R) shows complete genetic dominance to the sitter one (for^S). Rovers and sitters are found in nature at stable frequencies of 70% rovers and 30% sitters. The high frequency of both morphs along with the bimodality of the behavior suggests that natural selection may be acting to maintain these variants in nature. Accordingly, Sokolowski et al. (1997) showed that rovers have higher fitness in crowded environments, whereas sitters do better in uncrowded environments. Moreover, natural selection acts specifically on allelic variation at *for*.

The cloning of *for* demonstrated that it was identical to *dg2*, which encodes a *Drosophila* cGMP-dependent protein kinase (PKG) (Osborne et al., 1997). PKG enzyme activities and mRNA levels are higher in rovers than in natural sitters or sitter mutants. This suggests that PKG may be regulated differently in the two natural variants. *dg2* cDNA driven by the leaky expression of a heat shock promoter in transgenic sitter larvae changed larval behavior and PKG activity from sitter to rover, demonstrating rescue of the phenotype in transgenic flies. The research provides a behavioral function for PKG in naturally occurring variation and shows that subtle differences in PKG are sufficient to produce significant differences in larval foraging behavior.

The roles of PKG in cell signaling are not well understood (Wang and Robinson, 1997). One means by which PKG activities are increased in the cell is via nitric oxide (NO), which activates guanylyl cyclase, thereby increasing the intracellular level of cGMP. PKG is thought to be a major effector of cGMP; however, it is not involved in all intracellular cGMP signals. Three classes of cGMP binding proteins have been identified: cGMP-regulated phosphodiesterases, cGMP-gated ion channels, and cGMP-dependent protein kinases (PKG). Few substrates for PKG have been documented in the nervous system. A genetic dissection of foraging behavior should aid in uncovering novel PKG substrates.

We can speculate about how PKG acts in the fly to give rise to differences in foraging behavior by considering a simple working model in which foraging is divided into four components based on function: (1) monitoring of the external environment, (2) monitoring of the internal environment, (3) central processing of information, and (4) the behavioral output or response (Sokolowski and Riedl, 1999). PKG may be involved in one, several, or all of these components, and any combination of components may affect the outcome to be a rover or a sitter. Preliminary data on the expression patterns of *for* in the fly shows that it is expressed in subsets of tissues involved in olfaction, taste, gut, and central brain function. This closely parallels the expression of rat PKG1 found by Kroner et al. (1996), which was expressed in olfactory tissues (bulb and epithelium), the cerebral cortex, and the gut.

The following possibilities thus present themselves. First, PKG may be involved in sensing the external foraging environment, since rover/sitter behavioral differences are only exhibited in the presence of food. The level of PKG may differentially affect chemoreception in rovers and sitters, resulting in differences in how information about the foraging environment is interpreted. cGMP signaling is known to play a role in taste (Amakawa et al., 1990) and olfaction (Breer and Shepherd, 1993), both important elements of foraging. Increases in PKG showed attenuation of the response to stimulation by odorants in rat olfactory cilia (Kroner et al., 1996). Second, PKG function may be important for the internal feedback control of foraging. Feeding is influenced by the fullness of the gut, which sends hunger or satiety signals to the brain. NO–cGMP signaling has been implicated in neurotransmission in the vagus nerve involved in sending signals from the gut to the brain in mammals (Hatanaka et al., 1997). NO–cGMP–PKG signaling molecules along with neuropeptide Y (NPY) and leptin are expressed in the mammalian hypothalamus (Bhat et al., 1996; Kalra, 1997), known to be a key region of the mammalian brain involved in the regulation of food intake. Third, expression of *for* in regions of the central brain suggests that rover/sitter differences could also result from how the brain interprets and responds to foraging cues. PKG is likewise expressed in neural tissues (reviewed by Wang and Robinson, 1997), it may act to increase neural excitation, and it may play a role in long-term potentiation (Zhuo et al., 1994). Fourth, PKG may also be involved in the output or behavioral response. PKG has been implicated in the respiration and energy usage of skeletal muscle. Perhaps PKG levels may affect the interaction between central signals and the response generated in the muscles.

To summarize the speculations made above, PKG signaling may play a regulatory role at any one or combination of the elements of foraging behavior. An initial test of this working model could be done in flies by increasing the PKG level to that of rover only in a subset of all of the tissues where *for* is normally expressed. For example, one could express a rover copy of *for* in the gut alone, while every other tissue in the animal has the lower level of PKG found in sitters. This would enable us to address the hypothesis that internal signals arising from the gut alone give rise to rover behavior. Models of distributed function could be tested by expressing rover copies of *for* in combinations of tissues known to express PKG (e.g., the gut and central brain).

The third paper, published in *Cell* this month by de Bono and Bargmann (1998), also identifies two natural variants in the behavior of worms feeding and moving on a food source, a lawn of *E. coli*. Some wild-type strains are solitary foragers, moving across the food and feeding

alone, whereas others are social foragers, aggregating together on the food while they feed. More than 50% of social foragers are found in groups (ranging in size from three to several hundred worms), while less than 2% of solitary foragers are found in groups. Social foragers move twice as fast as solitary foragers in the presence of food. Like rovers and sitters in fly larvae, both types of worms show similar rapid speeds in the absence of food. The authors suggest that the social worms may aggregate due to the presence of mutually attractive, as yet unidentified stimuli. Solitary worms clump when food is limiting, indicating that the tendency to aggregate in worms, like foraging behavior in fly larvae, has both genetic and plastic components.

How was the gene for natural variation in worm foraging behavior identified? The authors gathered social strains of worms that arose from mutagenesis screens of the solitary forager strain in several labs. Genetic and molecular analysis of these mutant strains along with the wild-type social and solitary forager strains proved that the mutations in these strains were allelic to one another and to the wild-type social strain. Further genetic analysis showed that the differences in foraging behavior in wild-type and mutant worms was due to allelic variation at a single locus called *npr-1* for NPY receptor resemblance. Proof that social and solitary behavior resulted from variation at *npr-1* came from the findings that each of the three mutant strains had alterations in *npr-1* and that solitary behavior was restored in transgenic social worms with DNA from the open reading frame of the *npr-1* gene. NPR-1 expression, measured using a green fluorescent protein (GFP) reporter, was found primarily in the head but also in the ventral nerve cord, suggesting that NPR-1 likely functions in neurons to regulate behavior.

The remarkable finding was that a single amino acid substitution in *npr-1*, a valine to a phenylalanine substitution close to the transmembrane domain in the third intracellular loop of this seven transmembrane receptor, accounted for the difference between the natural variants. de Bono and Bargmann suggest that the single amino acid substitution found in these molecular isoforms alters NPR-1 receptor function and that this region of the protein may be important for the strength or specificity of G protein coupling. The presence of a valine or a phenylalanine in the protein was perfectly correlated to the behavior of wild strains (12 social and 5 solitary) collected from different parts of the world. To prove that the amino acid substitution was responsible for the differences in behaviors of the wild strains, they showed that the valine 215 *npr-1* transgene could efficiently confer solitary behavior onto a social strain, while the phenylalanine 215 *npr-1* transgene was inefficient in the same assay.

Thus, de Bono and Bargmann found two molecular isoforms in nature that differ in one amino acid and somehow affect behavior differently in natural populations. The studies further demonstrate that structural rather than regulatory differences in NPR-1 account for this behavioral variation in the wild. The authors speculate that the social/solitary polymorphism in worm foraging may have only evolved once over evolutionary time. If so, which of the forms—social or solitary—arose first during evolution remains to be determined. Although the *Cell* paper does not address the issue of the relevance of solitary and social foragers to life in the wild, it is likely that, as in the fly, these differences in behavior will have significant consequences for fitness.

By what mechanisms might NPR-1 act to modulate feeding behavior in worms? Consideration of the role of neuropeptides in other systems may provide some idea. Neuropeptides are found throughout the brain along with classical neurotransmitters. They are released from neurons and act through G protein–coupled receptors to modulate neuronal excitability by, for example, affecting ion channels, second messenger pathways, and enzyme activities. In most cases, neuropeptides regulate neuronal responses over longer time periods than do classical fast neurotransmitters. de Bono and Bargmann suggest that alterations in a neuropeptide could have significant long-lasting effects on behavioral performance and, in this way, could be involved in the generation of normal individual differences in behavior. In mammals, NPY, acting through the NPY receptors, has been shown to stimulate feeding and appetite; it also has anxiolytic and sedative activities (see references in Kalra, 1997; de Bono and Bargmann, 1998). Although NPY itself has not been found in worms, many other peptides are known to affect nematode activity and behavior. It is therefore possible that some other neuropeptide acts through NPR-1 to affect foraging behavior in worms.

The data on fly and worm foraging beg for comparison. Foraging behavior in both species is complex. It is characterized by differences in suites of behaviors whose expression is dependent on environmental factors (food quality and hunger levels). At the behavioral level, many parallels can be drawn between the rover/sitter foraging behavior in *Drosophila* larvae and the social/solitary foraging behavior of *C. elegans*. Rover larvae and social worms have longer foraging trails due to their higher speeds of movement than do sitter larvae and solitary worms when feeding in large homogeneous food patches. The behavioral differences in the worm and the fly larva are conditional on the presence of food (yeast and *E. coli*, respectively). In the absence of food (on agar), the fly and worm variants exhibit similarly rapid locomotion. The foraging behaviors of the fly and worm are plastic and can be modified by the environment. If a rover fly experiences a period of starvation or limited food, it shows more sitter-like behavior; similarly, solitary worms aggregate together as food is depleted. It is tempting to hypothesise that the behavior exhibited (rover or sitter, social or solitary) is a measure of how “motiviated” the animal is to forage. One difference between the organisms is that fly larvae do not form aggregations while foraging; when multiple larvae are in a dish, their distribution is random.

At the genetic level, the natural behavioral variants in the fly and worm are each attributable to a single major gene that alters the probability of behaving as one type or another in a given environment. Whether the gene products (PKG and NPR-1) are part of the same pathway remains to be determined. An NPY receptor has been cloned from flies (Li et al., 1992), and once mutants in this gene are generated, the function of this receptor in

fly foraging behavior should be tested. The involvement of cGMP in worm chemosensory transduction (Mori et al., 1996) and the recent cloning of worm guanylyl cyclase (Baude et al., 1997) suggest that the cloning of PKG from the worm should be next on the agenda. A link between NPY and PKG has been found in rat chromaffin cells, where NPY inhibits spontaneous fluctuations in $[Ca^{2+}]_i$ and activates a K^+ conductance through a PKG-dependent pathway (Lemos et al., 1997). However, NPY can also act through other signaling pathways. Whether NPY and PKG act together in neurons, and, if so, how they interact to affect foraging behavior are subjects for future investigations.

Finally, from an evolutionary perspective, if similar selective pressures were involved in the selection of rover/sitter and social/solitary foragers and their gene products are members of the same underlying biochemical pathway, then natural selection would have acted on different genes in the same pathway to produce similar behavioral variants in different species. The selection of different genes in the same pathway could result from chance events during evolution or different developmental constraints in the worm and fly resulting from the pleiotropy of most "behavioral genes." But the pleiotropy of behavioral genes is ample fodder for another minireview.

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