Systems biology meets behavior

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Early scientists were drawn to the study of insects in part because of the marvelous notion that their full behavioral repertoire was orchestrated from within. This was stated most succinctly perhaps by Spalding (1873): "When, as by a miracle, the lovely butterfly bursts from the chrysalis full-winged and perfect, . . .it has, for the most part, nothing to learn, because its little life flows from its organization like melody from a music box" (1). Of course, in time, environmental signals were discovered that modify insect behavior, making things more complex, and the dialectic between inherited and acquired behavioral traits arose. However, behavior is the ultimate arbiter of evolutionary change, so it has been a focus of organized biological studies for more than a century. The "Holy Grail" of understanding exactly where and how behavior is controlled, however, remains elusive. In PNAS, Chandrasekaran et al. (2) present behavioral analysis using tools from systems biology to reveal distinct neurogenomic states of transcriptional modules related to honey bee phenotypes. The heart of this unique work is that distinct bee behaviors across occupations are subserved by distinct neurogenomic states in the brain. These neurogenomic states that underlie different behaviors rely on transcriptional modules, some shared and some different. Because the connection distance between genes and behavior is large and complex, it is a surprise to find this level of predictability, especially because the data are from bees in their natural habitat.

Bee Behavior and the Genome

Tinbergen (3) famously identified key experimental questions distinguishing proximate (how) questions from ultimate (why) questions, setting the stage for behavioral analysis that brought it into the scientific fold. In this context, the honey bee is one of the best-studied organisms because of its intricate behavior and natural sequence of phenotypes. Von Frisch (4) identified the dance "language" that bees use to communicate the spatial location of food and other resources to their hive mates that has served as the basis of numerous analyses of the remarkable properties of the dance. Indeed, the common belief that bees and other insects are automatons belies their sophisticated behavioral repertoires. For

example, a bee goes through a regular progression of occupations, beginning as a worker, cleaning comb cells in the hive. Her next step is as a nurse, to feed honey to larvae and after that to feed nest mates. Finally, at approximately 3 wk of age, she begins foraging for pollen and nectar outside the hive. These transformations have been shown to correspond to changes in gene expression in a landmark study by Whitfield et al. (5),

> **Distinct bee behaviors across occupations are subserved by distinct neurogenomic states in the brain.**

who tracked gene expression as bees transitioned from nurses to foragers. Using microarrays to measure expression levels of ≈5,500 genes out of the ≈14,000 in the bee genome, they found that ≈2,000 had different levels of expression corresponding to their job.

Thus, the genome is an important source of information used to transition from one phenotype to the next. However, rather than the genome being a slavish "music box" playing its melody, bees can respond to the population dynamics of the hive. For example, Huang and Robinson (6) experimentally altered the distribution of phenotypes in a hive and could change the timing of the transitions among bee types. For example, adding older foragers to the hive radically slowed the transition of younger bees from nurses to foragers. Conversely, adding young bees to a hive accelerated that transition of young bees to foragers. Leoncini et al. (7) then showed that these changes occur via receipt of a fatty acid, ethyl oleate, produced by older foragers and seeming to be transferred with nectar gathered. How this chemical transforms behavior or gene expression is not known, but there are ≈10 known pheromones that can alter behavior in a hive, and it is known that the animals are responsive to key features of the current status of the hive.

Transcriptional Regulatory Network Related to Bee Behavior

Now, in a conceptual advance, Chandrasekaran et al. (2) use a systems ap-

proach to generate a transcriptional regulatory network (TRN) model to ask whether mRNA expression level patterns can predict behavioral states. Using samples from natural populations, they construct the TRN from expression levels measured under a variety of states, evaluate its performance in new test conditions, and then compare the subnetworks associated with the three chosen behavioral states: aggression, maturation, and foraging. Their approach is deceptively simple. Using a set of six steps, they develop a model that predicts gene expression from transcription factor expression. The most surprising outcome is how robustly the systems biology approach relates natural behaviors to transcriptional regulation. One might have imagined that the variance in natural behavior would overwhelm a highly structured systems approach. Because large-scale validation of the model using genetic interventions such as RNAi is not yet possible, the authors use comparative bioinformatics analyses to provide correlated information useful for interpreting their results. These analyses reveal that some factors such as hormones may drive global gene expression across the brain, and the high-level hubs in the model may provide useful entry points into understanding how regulation unfolds in time. Further, the model reveals numerous genes not previously known to regulate behavior.

Recently microarray analyses have begun to uncover genomic relationships to behavior in humans. Transcriptome analyses of the human brain (8) have shown the power of analyzing gene coexpression relationships in microarray data, revealing a genomic architecture previously unsuspected. Moreover, transcriptome organization between normal and autistic brains suggested differences in regional patterns in key brain regions between these two kinds of brains (9). These data suggest that convergent abnormalities of transcription and splicing may reflect common dysfunction characteristic of autistic brains.

The deeper question for the study by Chandrasekaran et al. (2) is how these distinct transcriptional states relate to the

control and regulation of behavior. Do they reflect behavioral choices made by existing neural circuits? Are they anticipating behavioral change by integrating environmental inputs and influencing neural circuits? How do genes and circuits collude to make the "music box" of Spalding? This study does not provide causal evidence but instead illuminates candidate signaling pathways controlling the interaction between genes and behavior.

Soon we can anticipate that such transcriptomic assessments of normal or pathological brains as related to behavior will be joined by analysis of message regulation by small RNAs and epigenetics. These additional levels of control will add more complexity to our understanding of how genes and the brain regulate and are regulated by behavior.

1. Spalding DA (1873) Instinct. With original observations on young animals. *Macmillan's Magazine* 27: 282–293.
2. Chandrasekaran S, et al. (2011) Behavior-specific changes in transcriptional modules lead to distinct and predictable neurogenomic states. *Proc Natl Acad Sci USA* 108:18020–18025.
3. Tinbergen N (1951) *The Study of Instinct* (Oxford Univ Press, New York).
4. Von Frisch K (1967) *The Dance Language and Orientation of Bees* (Harvard Univ Press, Cambridge, MA).
5. Whitfield CW, Cziko AM, Robinson GE (2003) Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302:296–299.
6. Huang ZY, Robinson GE (1992) Honeybee colony integration: Worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proc Natl Acad Sci USA* 89:11726–11729.
7. Leoncini I, et al. (2004) Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees. *Proc Natl Acad Sci USA* 101:17559–17564.
8. Oldham MC, et al. (2008) Functional organization of the transcriptome in human brain. *Nat Neurosci* 11: 1271–1282.
9. Voineagu I, et al. (2011) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474:380–384.