

drift excitability<sup>11</sup>. Ultimately, the significance of these findings for memory storage must be evaluated in the context of behavior, where there already exists considerable evidence for changes in neuronal excitability associated with learning and conditioning in many species<sup>10</sup>.

The plasticity of dendritic excitability naturally also has important consequences for subsequent dendritic processing of synaptic inputs. The suppression of A-type potassium channels can promote EPSP summation (compare ref. 11) and regenerative synaptic interactions in dendrites, and thus enhance the impact of EPSPs on somatic output, as expected in models of E-S potentiation. Furthermore, the enhancement of the back-propagating AP can also lower the threshold for triggering dendritic calcium spikes, which

can lead to axonal burst generation<sup>3</sup>. In this way, the enhancement of dendritic excitability associated with LTP provides a means of further amplifying the impact of synaptic plasticity on neuronal output.

Finally, in a wider sense, the study by Frick and colleagues is significant because it links three flourishing fields at the heart of cellular and systems neuroscience: LTP, intrinsic plasticity and dendritic excitability. Examining the mechanisms and functional consequences of this dendritic marriage between synaptic and intrinsic plasticity promises to lead to a deeper understanding of memory storage and dendritic function in the brain.

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## Taking sides in the nervous system with miRNA

Piali Sengupta

The mechanisms by which bilateral asymmetry is established in the nervous system are poorly understood. A recent report in *Nature* describes a novel microRNA that mediates left–right asymmetry in a pair of *C. elegans* chemosensory neurons.

At first glance, the nervous systems of vertebrates and invertebrates seem bilaterally symmetrical, but on closer inspection left–right asymmetries become apparent. Humans, for example, show gross anatomical differences between right and left temporal lobes, and visual and language faculties are asymmetrically distributed between the two hemispheres. How these asymmetries arise during development remains something of a mystery (for review, see ref 1). In the nematode *Caenorhabditis elegans*, the AWC and ASE chemosensory neuron pairs are bilaterally symmetrical based on anatomical considerations, but nevertheless display asymmetrical gene expression patterns. A recent study in *Nature* by Johnston and Hobert identifies a microRNA (miRNA) as a crucial mediator of this asymmetry in the ASE neurons<sup>2</sup>.

Of the 302 neurons in the *C. elegans* hermaphrodite, 32 neurons, including 14 bilateral pairs, comprise the chemosensory system. The ASE pair mediates responses to water-soluble compounds<sup>3</sup>. Despite expressing a set of genes in common, having similar morphologies and connecting to identical sets of postsynaptic

partners, the left and right ASE neurons (ASEL and ASER) are not identical. ASEL alone expresses the transmembrane guanylyl receptor cyclase genes *gcy-6* and *gcy-7* and responds to sodium, whereas ASER expresses *gcy-5* and primarily responds to chloride and potassium<sup>4,5</sup>. *C. elegans* apparently maximizes the functionality of its limited repertoire of chemosensory neurons by further lateral differentiation.

Earlier this year, the Hobert laboratory showed that the ASEL/R asymmetry is mediated via competition between two protein complexes present in both ASEL and ASER neurons<sup>6</sup>. One complex contains the OTX-like homeodomain protein CEH-36 and the transcriptional coactivator LIN-49, and the other contains the Groucho-like transcriptional corepressor UNC-37 and the NKX6-like homeodomain protein COG-1. The outcome of this competition is biased by the expression of COG-1, which is present at far higher levels in ASER than in ASEL neurons<sup>6</sup>. Thus, the COG-1/UNC-37 complex outcompetes the CEH-36/LIN-49 complex in ASER neurons to repress ASEL-specific genes, thereby allowing the expression of ASER-specific genes. In contrast, low levels of COG-1 in ASEL allow the CEH-36/LIN-49 complex to activate the expression of ASEL-specific genes. Of course, this result immediately raised the question of

how COG-1 expression is regulated in ASEL and ASER neurons.

Enter the *lisy-6* miRNA. The miRNAs are small (~21 to 22-nucleotide) RNAs, which are processed from larger RNAs with extensive secondary structure (see ref. 7 for review). These small RNAs downregulate gene expression by base-pairing with cognate complementary sequences in the 3' untranslated regulatory regions (UTR) of target mRNAs, thereby preventing their translation. The first miRNA was identified over a decade ago in *C. elegans*<sup>8,9</sup>, but only with the recent identification of conserved miRNAs in other multicellular organisms have miRNAs evolved from being a nematode-specific oddity to representing the newest general mechanism of gene regulation. Genome mining has predicted 100–300 miRNAs in different species, and several of these are expressed in a tantalizing tissue- and developmental stage-specific manner<sup>7</sup>. However, to date, the functions of only a handful of these miRNAs have been defined, and few targets have been identified *in vivo*.

Johnston and Hobert identified *lisy-6* in their screen for mutants affecting ASEL/R asymmetry. In *lisy-6* mutants, ASEL neurons adopt ASER-like properties, whereas shared ASE-like characteristics and ASER identity are unaffected. The authors could rescue the *lisy-6*

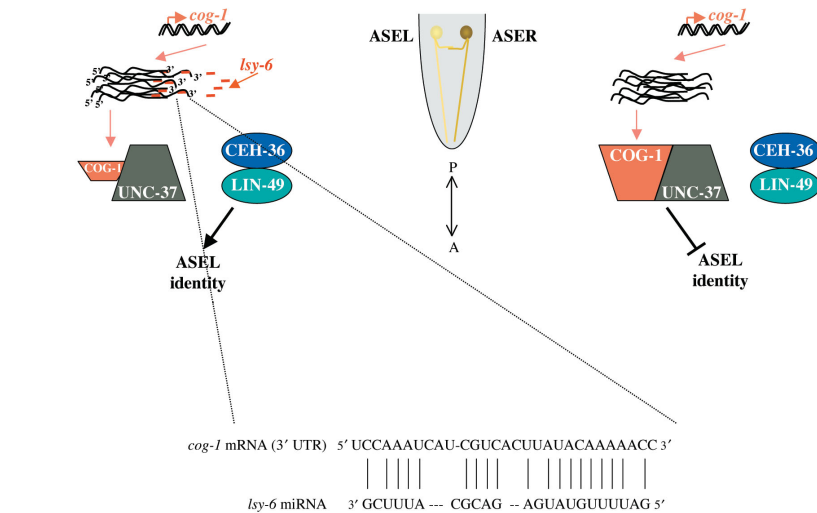
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phenotype with a fragment that did not encode a protein product but instead encoded an RNA with a predicted hairpin structure. Within this hairpin lay a 23-nucleotide sequence that was highly conserved in the related nematode *C. briggsae* and likely was an miRNA. The authors used several approaches to confirm that it was indeed this miRNA that represented the *lgy-6* locus. Most convincingly, they showed that a mutation that disrupted the hairpin structure also disrupted rescue of the *lgy-6* phenotype, and moreover, that a compensatory mutation that restored the secondary structure also restored the rescuing ability of the gene. The authors found that *lgy-6* was expressed in ASEL but not ASER neurons, and that misexpression of *lgy-6* in ASER neurons was sufficient to induce ASEL characteristics.

How does *lgy-6* act? In *lgy-6* mutant animals, ASEL neurons expressed COG-1 at levels similar to those of ASER neurons. Interestingly, the 3' UTR of the *cog-1* mRNA contained sequences that were complementary to *lgy-6*. Proof that *cog-1* was indeed the target of *lgy-6* was provided by demonstrating that expression of a 'sensor' containing *cog-1* 3' UTR sequences fused to the coding sequences of GFP was brought under *lgy-6* control, and that mutations in the *lgy-6* complementary site abolished this regulation. This result suggests that levels of COG-1 are regulated by *lgy-6* post-transcriptionally in ASEL neurons (Fig. 1). The authors previously also observed regulation of COG-1 at the level of transcription<sup>6</sup>; however, this may occur simply because COG-1 autoregulates to maintain its expression (O. Hobert, personal communication). Thus, the *lgy-6* miRNA may act to 'asymmetrize' initially symmetric *cog-1* expression, leading to different subtype identities in ASEL and ASER neurons.

This satisfying story raises several intriguing issues. The *lgy-6* miRNA was not identified in any of the extensive cloning or bioinformatics sweeps to identify all miRNAs predicted to be encoded by the *C. elegans* genome<sup>10–12</sup>. The *lgy-6* sequences are conserved in *C. briggsae*, which is separated from *C. elegans* by 50–100 million years of evolution, although the authors do not report whether *lgy-6* is asymmetrically expressed and shows conserved function in ASE neurons of *C. briggsae*. It is also unclear whether *lgy-6*-related sequences are found in other animals. However, the identification of *lgy-6* raises the real possibility that there may be many more miRNAs yet to be identified and characterized.

This study yet again highlights the power of forward genetics in elucidating gene, or in this case miRNA, function. One issue with study-



**Figure 1** How *lgy-6*-mediated asymmetry determines ASEL/R subtype identities. The ASEL and ASER chemosensory neurons acquire distinct subtype identities as a consequence of an antagonistic interaction between the COG-1/UNC-37 and CEH-36/LIN-49 protein complexes. This competition occurs through asymmetric expression of the NKX6-like homeodomain transcription factor COG-1. Higher levels of COG-1 in ASER neurons results in repression of ASEL-specific genes and expression of ASER-specific genes, whereas lower levels of COG-1 in ASEL neurons allows the CEH-36/LIN-49 complex to promote the expression of ASEL-specific genes. COG-1 levels are downregulated specifically in ASEL neurons by ASEL-expressed *lgy-6* miRNA. This miRNA binds to a complementary site in the 3' UTR of the *cog-1* mRNA to prevent translation (inset).

ing miRNA function has been that because miRNAs are so small, generating mutations via targeted reverse genetics approaches has proved to be technically challenging. Although it is possible that further iterations of bioinformatics software might yet identify *lgy-6*, given its fairly esoteric role, it is highly unlikely that its functions would have been easily identified via reverse genetics. In addition, in animals (as opposed to plants), miRNAs bind to target sequences that are not perfectly complementary<sup>7</sup>, making the identification of target genes *in silico* extremely difficult. Individual miRNAs appear to regulate multiple target genes, suggesting that miRNA-mediated post-transcriptional regulation of gene expression may become the norm instead of the exception.

The identification of *lgy-6*-mediated regulation of L/R asymmetric gene expression is a significant advance, but the signal that underlies asymmetric expression of *lgy-6* itself has yet to be discovered. When and where does this signal act? Are all cases of *C. elegans* nervous system asymmetry mediated via the same signal? Do similar signals act in other organisms? Genetic screens using the asymmetrically expressed *lgy-6* as a marker may answer some of these questions.

Finally, this study defines the first miRNA function in the nervous system of any organism. The *let-7* miRNA downregulates the hunchback-related gene *hbl-1* in the nervous

system in *C. elegans*, but the functional consequences of this regulation are unclear<sup>13,14</sup>. Might miRNAs have different or unexpected functions in the nervous system as opposed to other tissues? Probably not. However, given that nervous system function critically depends on thousands of neuronal subtypes that must be generated in a strict temporally and spatially regulated manner, it is likely that the problem of regulating gene expression in the nervous system is especially complex. In *Arabidopsis*, most miRNA targets are believed to be transcription factors involved in pattern formation and cell differentiation<sup>15</sup>. Thus miRNAs are excellent candidates to determine cellular diversity in the nervous system. The exploration of miRNA functions is in its infancy. The Johnston and Hobert paper<sup>2</sup> provides a first taste of the many surprises that miRNAs have in store for us in the future.

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# Listening to your heart: interoceptive awareness as a gateway to feeling

Antoine Bechara & Nasir Naqvi

**Does awareness of the internal state of one's body relate to the conscious experience of feeling? A new imaging study suggests that body awareness mediated by activity in the anterior insular cortex may contribute to the intensity of negative emotions.**

When you see the person you are in love with, your heart may race, your skin may flush, and your facial muscles may contract in a smile. You may also hear your heartbeat or sense 'butterflies' in your stomach. In addition, you experience feelings of love and elation directed toward your loved one. Without experimental evidence, James and Lange<sup>1</sup> suggested that feelings are the consequence of these body sensations, but philosophers have argued that the two differ because they have different objects. Body sensations involve awareness of the body's internal state; feelings are directed toward objects in the external world. Damasio<sup>2,3</sup> has argued, however, that emotional feelings require the two objects—the body, which provides a substrate for feeling, and the external object that triggers the body changes in the first place, and toward which the feeling is directed.

Critchley and colleagues<sup>4</sup> now provide data suggesting that the subjective experience of emotions results from brain activity caused by such body states. Using functional magnetic resonance imaging (fMRI) and voxel-based morphometry (which estimates the size of a brain region), they identified brain areas engaged when subjects tried to sense whether their heartbeat was in sync with a series of tones. The size and activity of the right anterior insular cortex were related to individuals' accuracy in sensing the timing of their own heartbeats. Activity in this region was also correlated with an individual's propensity to subjectively experience certain emotions.

These findings provide important validation of the theoretical view of James and Lange<sup>1</sup> that neural systems supporting the perception of body states are a fundamental ingredient in the subjective experience of emotions. They also support Damasio's<sup>2,3</sup> and Craig's<sup>5</sup> view that the

right anterior insular cortex is important in mapping body states into feelings.

In one aspect, however, the findings depart slightly from the view of Damasio<sup>2,3</sup> that feelings arise in conscious awareness through a second-order representation of body changes in relation to the object or event that initiated them. Damasio distinguishes emotions from feelings. Emotions are changes in body and brain states triggered by a dedicated brain system that responds to the content of one's perceptions, actual or recalled. Body responses range from changes in heart rate or smooth muscle contraction to changes perceptible to an external observer (such as those to posture or facial expression). The signals generated by these body responses produce brain changes that are perceptible mostly to the individual and provide the essential ingredients for what is ultimately perceived as a feeling. Thus emotions are what an outside observer can see; feelings are what the individual subjectively experiences.

An emotion begins with appraisal of an emotional stimulus, such as the person you love. Even after brief presentation, signals evoked by that stimulus are carried from sensory areas to a number of emotion-triggering sites elsewhere in the brain, including the amygdala and orbitofrontal cortex (Fig. 1). There may be differences in how these regions process emotional information: the amygdala is more engaged in triggering emotions when the emotional stimulus is present; the orbitofrontal cortex is more important when it is recalled from memory<sup>6</sup>. To create an emotional state, activity must propagate to execution sites, which include the hypothalamus, the basal forebrain and nuclei in the brainstem tegmentum (Fig. 1).

Feelings result from neural patterns that represent changes in the body's response to an emotional stimulus. Representations of these body states are formed in visceral sensory nuclei in the brainstem, insular cortex and lateral somatosensory cortex (SII and SI).

Probably the cortical representation (rather than brainstem activity) produces conscious feelings. Damasio and Craig agree that the right anterior insular cortex is important in mapping visceral states and in bringing interoceptive signals to conscious perception. However, Craig suggests that this region also translates the visceral states into subjective feeling and self-awareness. In Damasio's view, a first-order mapping of 'self' is supported by brainstem regions, insular cortex and somatosensory cortex. However, additional regions, such as thalamus and anterior cingulate cortex, are required for second-order mapping of the relationship between organism and emotional object and for integration of information about the body with information about the world.

Critchley and colleagues<sup>4</sup> aimed to isolate a component of feeling, namely the mapping of the visceral state. They scanned the brains of subjects with fMRI during a heartbeat detection task. In half the trials, subjects tried to determine whether a series of notes occurred in sync with their heartbeat (an interoceptive event). In the other half, they were asked whether one of the notes had a different pitch than the rest (exteroceptive). Subjects were rated on validated clinical questionnaires that reflect anxiety, depression and other emotional states. Finally, using voxel-based morphometry, the authors measured the size of a region of interest, the insular cortex.

They found that focusing awareness on heartbeat timing, as opposed to note pitch, increased neural activation in anterior insular cortex, lateral somatosensory cortex and dorsal anterior cingulate cortex. Most importantly, subjects' accuracy in detecting their heartbeats correlated with both activity and gray matter volume of right anterior insular cortex. Self-report measures of anxiety (and to some extent other negative emotions) correlated with accuracy of heartbeat detection and activity in right anterior insular cortex. The authors conclude that individual differences in the ability to per-

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