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and, more importantly, correlated with behavior, as a low dose insufficient to induce unconsciousness also failed to significantly increase VLPO activity. In addition, VLPO lesions in mice produced an acute resistance to isoflurane-induced unconsciousness, further suggesting that VLPO neurons are a target for isoflurane. However, while *c-Fos* and lesion data may be suggestive of a mechanism, they are not definitive.

The highlight of this paper [2] is the electrophysiological work using hypothalamic slices. The authors report that isoflurane selectively depolarized NA(–) cells, the subtype of VLPO neurons thought to promote sleep. Surprisingly, the excitatory effects of isoflurane were extrasynaptic. In other words, rather than indirectly activating VLPO neurons through synaptic inputs, isoflurane *directly* depolarized VLPO neurons. The authors go a step further and show that closure of a background potassium conductance is the likely mechanism.

The vast majority of electrophysiological work to date has found that anesthetics enhance inhibitory receptors or inhibit excitatory receptors, and in either case the common end result is neuronal depression. Although direct activation of central respiratory chemoreceptor neurons by isoflurane has been previously reported [20], direct anesthetic-induced depolarization of neurons is a highly unusual finding. More importantly, the discovery that isoflurane selectively activates sleep-promoting NA(–) neurons in the VLPO supports an emerging concept that anesthetics contribute to unconsciousness through specific effects at discrete neural circuits that regulate sleep and wakefulness.

Future Directions

The mechanisms of general anesthesia have evolved from an elusive pharmacology puzzle to a tractable neuroscience problem. Despite considerable advances in our knowledge of the molecular effects of anesthetics, much remains to be learned about the profound and complex changes that occur at the level of neural circuits and systems during general anesthesia. With our current knowledge of the molecular mechanisms of general anesthesia and the neurobiology of arousal, it has become feasible to apply a systems neuroscience analysis to the problem [17]. Such analyses will provide a framework to explore and better understand the mechanisms of general anesthesia.

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Plant Development: How Long Is a Root?

The plant hormone cytokinin controls root growth by balancing the division and differentiation of stem cells. But what controls accumulation of cytokinin? A new study has identified a regulatory loop between a transcription factor, *PHABULOSA*, and cytokinin biosynthesis that creates robust domains of cytokinin activity.

Anthony Bishopp[1], Robertas Ursache[2], and Ykä Helariutta[2,*]

Root growth is a highly variable process that is dependent on environmental stimuli. It is coordinated by a small population of undifferentiated stem cells close to the root tip (known as the meristem). These cells undergo several rounds of cell division before they elongate and finally differentiate [1]. The rate of root growth and the size of the meristem are

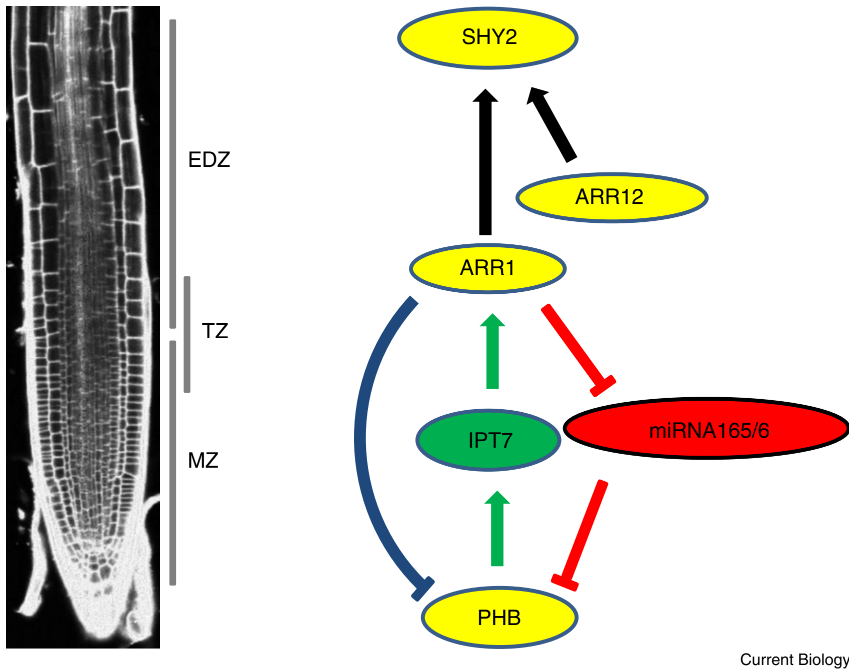

Figure 1. The long and short of it: mechanisms for controlling root length.

(Left) The growing *Arabidopsis* root can be separated into three overlapping regions. In the meristematic zone (MZ) cells are competent to divide. After several rounds of division, daughter cells enter an elongation/differentiation zone (EDZ). The point at which cells start to elongate varies between the different cell lineages and has been broadly classified as the transition zone (TZ). (Right) Schematic model showing the regulatory network controlling the interplay between cytokinin and PHABULOSA. (Root image provided by Shunsuke Miyashima.)

determined by the collective output of cell division, cell elongation and cell differentiation (Figure 1A).

More than 50 years ago, researchers identified the plant hormone cytokinin as a factor inhibiting root growth [2]. Cytokinin has since been shown to mediate this effect by controlling the size of the meristem [3–5]. However, only now are we beginning to understand how a stable zone of cytokinin is maintained in the root meristem. In a recent issue of *Current Biology*, Dello Ioio and colleagues [6] report that the expression of *IPT7* (a rate-limiting component of the cytokinin biosynthesis pathway) is directly regulated by the transcription factor PHABULOSA (PHB). This activation occurs in the context of an elegant regulatory loop with *PHB* and the authors propose that through this, cytokinin limits its own activity. They provide convincing support for this concept using a combination of experimental techniques and mathematical simulations.

PHABULOSA is a member of the class III HD-ZIP gene family. In *Arabidopsis*, this family is represented by five members that have overlapping roles in the specification of organ polarity and patterning [7,8]. All five genes are expressed in the root meristem and act as dosage-dependent regulators to specify vascular cell identity in roots [9]. Dello Ioio *et al.* [6] observed that the two class III HD-ZIP proteins PHABULOSA and PHAVOLUTA (PHV) also control meristem size. They observed that the heterozygous dominant gain-of-function alleles *phb-1d*/+ and *phv-1d*/+ develop short roots and smaller meristems. In these mutant alleles the *PHB* or *PHV* transcripts are rendered resistant to regulation by microRNA165/6 and the domains in which they are expressed become broader [9–11]. To test whether these genes were required in regulating meristem size, Dello Ioio *et al.* [6] observed that in the double loss-of-function *phb phv* mutant, plants had longer roots with larger meristems. But how do *PHB* and *PHV* fit into the existing framework regulating meristem size?

The phenotypes that Dello Ioio *et al.* [6] observed in the *phb1d*/+ and *phv1d*/+ gain-of-function alleles closely resemble those of plants supplied with exogenous cytokinin; the *phb phv* loss-of-function mutant closely resembles lines in which cytokinin signalling is impaired [3]. They examined several conditions in which cytokinin levels or signalling were altered and observed that the meristem phenotypes of the gain-of-function mutants could be suppressed when the cytokinin response regulator *ARR1* was knocked out. They also observed that supplying exogenous cytokinin could complement the meristem size phenotype of the loss-of-function *phb phv* mutant. Together these results neatly demonstrate that PHB/PHV act to control meristem size by controlling cytokinin action, most likely through modulating cytokinin synthesis.

The idea of a group of specific transcription factors promoting the site-specific biosynthesis of cytokinin is very attractive. One source of cytokinin in the root meristem is via transport through the phloem. While this is important in certain processes, such as vascular patterning in the root meristem [12], degradation of phloem-derived cytokinin has no bearing on meristem size [13]. Could PHB/PHV promote cytokinin biosynthesis to generate the local supply of cytokinin required to regulate root growth?

To investigate this, Dello Ioio and colleagues [6] focused on the isopentyl transferase gene family (IPT). Cytokinin synthesis requires multiple steps, but the rate-limiting step is catalyzed by the IPTs [14,15]. They discovered that expression of *IPT7* is reduced in *phb phv* and increased in both the *phb1d*/+ and *phv1d*/+ gain-of-function mutants. Crucially, they noted that *IPT7* is a direct target of PHB. The key experiment came when they miss-expressed *IPT7* in the *phb phv* double mutant, and were able to completely restore the wild-type root length and meristem size. Coupled with the observation that meristem size is indistinguishable in *ipt7, phb phv* and *phb phv ipt7*, this shows convincingly that *PHB* controls root growth through modulating cytokinin biosynthesis.

How does this fit in with the existing knowledge of cytokinin action? In

addition to cytokinin, the hormone auxin also plays a pivotal role in specifying the zone where cells differentiate [3,16]. This coordination is achieved through the activation of the auxin response inhibitor *SHY2* by the cytokinin response regulators *ARR1* and *ARR12* [17,18]. The cytokinin-mediated expression of *SHY2* negatively regulates the expression of the PIN class of auxin transporters to restrict the domain of auxin output and promote cell differentiation [17,18]. Additionally, *SHY2* represses the cytokinin biosynthesis gene *IPT5* to create a negative feedback loop [17].

It is known that cytokinin action is self-limiting and feeds back on the expression of IPTs after a certain threshold [14]. The new data presented by Dello Ioio *et al.* [6] show that this mechanism could be achieved partly through *PHB*. *PHB* expression is rapidly reduced when plants are supplied with exogenous cytokinin. In addition to the transcriptional repression of *PHB*, Dello Ioio *et al.* also observed that cytokinin represses the expression of microRNA165A. As microRNA165/6 has previously been shown to function in the post-transcriptional repression of *PHB* [9,11], the authors propose that this mechanism provides a feed-forward loop to regulate PHB activity. Such a loop comprises three components; the first component (ARR1) regulates the activity of the second component (PHABULOSA) by two means (Figure 1B). On one hand ARR1 represses the transcription of *PHB* (shown in Figure 1B in blue); and on the other hand ARR1 represses the expression of a third component (microRNA165/6) that exerts post-transcriptional repression of *PHB* (shown in red). PHB promotes the biosynthesis of cytokinin (via IPT7), which activates ARR1 (shown in green). This provides a molecular circuit whereby cytokinin both represses *PHB* and prevents the repression of *PHB*, which in turn feeds back on the synthesis of the original signalling molecule, cytokinin. The authors refer to this system as an incoherent feedforward loop.

But why would such a complicated regulatory network be required to restrict the spatial domain of cytokinin? In order to answer this question, Dello Ioio *et al.* [6] ran a series of mathematical simulations of this molecular network with, and without, the regulation of microRNA165/6 by cytokinin. They discovered that this regulatory loop dampens the reduction and accelerates the recovery of *PHB* levels as cytokinin levels fluctuate. This network is therefore likely to provide a robust mechanism that can position the boundary between cell division and cell differentiation to determine meristem size.

In summary, the work presented by Dello Ioio *et al.* provides a complex yet elegant molecular network to provide stable levels of cytokinin. Cytokinin action then determines the point at which cell differentiation occurs, thereby controlling the size of the root meristem as well as the overall growth of the root. One truly exciting aspect about this work is that many of the same components regulate other aspects of root development. For example, auxin, cytokinin, *PHB* and microRNA165/6 all regulate vascular pattern formation [9,13]. Auxin and cytokinin control root and shoot branching [19,20]. It will be fascinating to see if similar molecular networks also specify the positioning of cytokinin output during these developmental processes.

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