cellular functions. The data presented by Nutt et al. (2005) demonstrate that nutrient deprivation is, instead, directly linked through caspase-2 to the apoptotic machinery and that active suppression of this pathway by continuous glucose metabolism is required for survival. The fact that this pathway can be activated due to age-related changes also suggests that metabolic decline may contribute directly to cellular and organismal aging by inducing caspase activation.

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VAV's Got Rhythm

Biological rhythms with periods of less than a day are physiologically important but poorly understood. In this issue of *Cell,* Norman, Maricq, and colleagues (Norman et al., 2005) show that VAV-1, a guanine nucleotide exchange factor for Rho-family GTPases, is necessary for three rhythmic behaviors in the nematode *Caenorhabditis elegans*: feeding, defecation, and ovulation.

Rhythmic processes are a fundamental and fascinating aspect of animal life. Ultradian rhythms, which have a period of less than a day, are widespread and include the pumping of the heart, breathing, gut peristalsis, metabolism in yeast, and courtship song in the fruit fly *Drosophila*. Whereas rhythms with a longer period, such as circadian rhythms, are very dependent on changes in gene regulation and expression, those with a shorter period will depend more on changes in cellular physiology and signaling pathways. Exactly how such rhythms are orchestrated remains unclear. In this issue of *Cell*, Norman et al. (2005) identify an exciting new link between calcium signaling and Rho GTPases in the machinery regulating rhythmic behavior in the nematode *Caenorhabditis elegans*.

C. elegans is a surprisingly rhythmic creature; even its sinusoidal movement has more than a hint of salsa to it. Less glamorous—though none the less intriguing—are the rhythms that control feeding, defecation, and ovulation, three processes at the heart of this animal's life. These rhythms have a range of periods. For example, on average, the pharynx pumps every 250 ms when food is present, whereas the contraction of the gonadal-sheath cells (required for ovulation) and defecation occur less frequently, on average every 7 s and 45 s, respectively. In each case, a neuronal input is not required to maintain the rhythm, although both environmental and neuronal inputs do regulate the rhythms.

The presence of three rhythmic processes with differing functions and periodicity raises the question of whether these processes share common molecular mechanisms. This is indeed the case. It is now clear that each of these three rhythms is regulated by the second messenger inositol 1,4,5-trisphosphate (IP_3) and its receptor, ITR-1 (IP_3R) (Clandinin et al., 1998; Dal Santo et al., 1999; Walker et al., 2002; Yin et al., 2004). IP_3 acts on IP_3Rs, calcium channels located in the endoplasmic reticulum, to regulate intracellular calcium signals, which are often oscillatory (Berridge et al., 2003). How the IP_3/Ca^{2+} signals are regulated in these rhythms is a central question. Now, Norman et al. (2005) have identified VAV-1 as a common upstream component of IP_3 and/or Ca^{2+} signaling in all three rhythms in the worm.

Vav-1 is a guanine nucleotide exchange factor (GEF), which activates the Rho family of small GTPases by promoting the exchange of bound GDP for GTP. Rho GTPases are well known for their roles in the regulation of the cytoskeleton, cell polarity, and cell division. However, Vav is also a proto-oncogene with a role in T cell development, activation, and calcium signaling (see Tybulewicz, 2005 for review). In the current study, deleting the *vav-1* gene in *C. elegans* results in growth arrest at the first larval stage (L1), a phenotype often associated with a disruption of feeding (Norman et al., 2005). In fact, pumping of the pharynx in these animals is severely disrupted. The authors rescue the feeding defect by expressing VAV-1 under the control of pharyngeal-specific promoters, allowing the animals to grow into viable adults. In so doing, they also reveal essential functions for *vav-1* in both ovulation and defecation. Importantly, both the pharynx rescue experiments and mosaic analysis of the defecation phenotype localize *vav-1* function to the pharyngeal muscle and intestinal cells rather than to the nervous system.

What lies upstream of *vav-1*? In mammalian cells, the GEF activity of Vav is regulated by tyrosine phosphorylation (Tybulewicz, 2005). Experiments in which specific VAV-1 mutants are expressed in body-wall muscle (a tissue that does not normally express VAV-1) demonstrate that changing the equivalent tyrosine residues in *C. elegans* VAV-1 also alters the function of this protein (Norman et al., 2005). Furthermore, we know that, dur-

ing ovulation, an EGF receptor (RTK) homolog called LET-23 and its ligand LIN-3 act upstream of the IP_3 receptor, ITR-1. Genetic analysis clearly places *vav-1* downstream of *let-23* and upstream of *itr-1* in the gonad. This suggests that tyrosine kinase activity may link cell-surface signals to VAV-1 activity and thus to Ca^{2+} release. It is important that we now clarify the signals upstream of VAV-1 in each tissue and, in particular, establish whether similar tyrosine-kinase-dependent mechanisms exist in the pharynx and intestine. Such studies will define to what extent there is a common upstream mechanism for the rhythmic processes of *C. elegans* and should illuminate the contributions of intracellular and intercellular signaling.

What lies downstream of VAV-1? Genetic analysis puts *vav-1* upstream of *itr-1* in the gonad and intestine, thus placing VAV-1 into the IP_3-mediated signaling pathway in at least two tissues. A particularly gratifying aspect of the Norman et al. paper is the combination of molecular and genetic approaches and in vivo imaging. In both the pharynx and the gut, perturbing VAV-1 expression results in disruption of the oscillating calcium signals associated with the rhythmic behaviors. Thus, VAV-1 is clearly linked to IP_3 and calcium signaling. Norman et al. (2005) show that the ability of VAV-1 to regulate Rho GTPases is also important. A GEF-defective transgene, unlike its wild-type counterpart, was unable to rescue the pharyngeal pumping phenotype. Furthermore, using a *vav-1* gain-of-function mutant in the worm intestine, the authors show that *vav-1* GEF activity and small GTPase activity are linked in the control of defecation. However, determining which of the *C. elegans* Rho GTPases is acted upon by VAV-1 in each cell type is more challenging, as these proteins have major roles in development and cell division. Nevertheless, Norman et al. show that *rho-1* is necessary for normal ovulation, whereas *rho-1*, *ced-10* (Rac), and *mig-2* (Rac) are involved in defecation. Thus, GEF activity and Rho GTPases are linked in the intestine, and either GEF activity or Rho GTPases are important in the pharynx and gonad.

One important question that we now need to answer is how VAV-1 and the small GTPases regulate Ca^{2+} levels in these cells. Of course, Rho-family GTPases have many functions that could regulate Ca^{2+} signals as well as other aspects of cell physiology to coordinate these rhythms in the worm. It is also known that Vav activates phospholipase Cγ (PLCγ) in mammalian cells and thus stimulates IP_3 production. PLCγ, encoded by the *plc-3* gene, is already known to be important in ovulation, defecation, and feeding (Yin et al., 2004; Espelt et al., 2005; H.A.B., unpublished data). The exact mechanism by which Vav activates PLCγ in T cells is unclear, but it may involve PI3K, a lipid kinase that does not appear to be involved in the three rhythms in *C. elegans.* Alternatively, Rho and Rac are also known to regulate phosphatidylinositol 4-phosphate 5 kinase (PIP5K) and thus phosphatidylinositol (4,5)bisphosphate (PIP_2) production in mammalian cells. PIP_2 is the precursor of IP_3, and so its availability might regulate IP_3-mediated rhythms. Norman et al. show that *ppk-1*, a PIP5K homolog, is required for the defecation cycle and acts downstream of *vav-1.* They suggest that regulation of *ppk-1* expression and PIP_2 levels may be a mechanism by which VAV-1 regulates calcium signals (see Figure 7F in Norman et al., 2005). However, PIP_2 could have a broader role. Binding of Vav to PIP_2 (and PIP_3) via the pleckstrin homology domain regulates its GEF activity in mammalian cells (Han et al., 1998). In addition, PIP_2 has many functions, including the regulation of IP_3Rs (Lupu et al., 1998) and other ion channels. In fact, a variety of ion channels are known to be involved in each of the three rhythmic behaviors. Thus, PIP_2 has the potential to affect the localization and function of many components of the mechanism that regulates rhythmic behaviors in the worm. The fact that VAV-1 may regulate PIP_2 levels and be regulated by them should make unraveling these pathways a stimulating challenge.

The work of Norman et al. extends our knowledge of the networks that regulate the ultradian rhythms of organisms and adds further support for the existence of a shared core molecular system. Whether this system operates in exactly the same way in each tissue remains unclear. For example, in the pharynx of *C. elegans*, partial ablation of IP_3 signaling results in a failure to upregulate pumping in response to food but does not abrogate the ability of the animals to pump when they are given an alternative stimulus—in this case, the neurotransmitter serotonin (Walker et al., 2002). In contrast, *vav-1* mutants have severely disrupted pharyngeal pumping even in the presence of serotonin (see Figure 2 in Norman et al., 2005). On the other hand, *itr-1* (Dal Santo et al., 1999) and *vav-1* (see Figures 5 and 7 in Norman et al., 2005) are clearly required for defecation, and no stimuli that circumvent these requirements have been identified.

Many genes have been identified that influence either one or more ultradian rhythms, but how these genes interact to form oscillators and the environments that regulate them remain to be elucidated. There are a number of key questions that need to be addressed regarding how the oscillators are established and how their properties (that is, period and variability) are determined. The fact that VAV-1 is involved in several different ultradian rhythms in *C. elegans* suggests that this mechanism will be found in other animals.

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