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Protons as Intercellular Messengers

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Muscle contractions are driven by neurotransmitters released at neuromuscular junctions. In this issue, Beg et al. (2008) report that protons, in the absence of neurotransmitters and neurons, can stimulate muscle contractions involved in the defecation cycle of the worm *Caenorhabditis elegans*. Their results identify protons as a new intercellular messenger and suggest that proton-mediated intercellular communication may be a widespread phenomenon.

Protons are essential ubiquitous ions that regulate basic biological processes such as pH homeostasis and the control of cell volume (Mahnensmith and Aronson, 1985). Under normal physiological conditions, cells are protected from increased acidity. Exposure of cells to highly acidic environments is often associated with stimuli such as acute sensations and the sensation of pain (nociception) or pathological states such as ischemia, seizure, and cancer.

In addition to these housekeeping roles, the involvement of protons in modulating the function of the central nervous system has long been suspected. Low pH solutions can induce action potentials (Vukicevic and Kellenberger, 2004) and evoke cation currents in hippocampal neurons (Wemmie et al., 2002). An acid-sensing proton-gated cation channel (ASIC), which is localized to the postsynaptic termini of hippocampal synapses, modulates dendritic spine density (Zha et al., 2006) and is also implicated in learning and memory (Wemmie et al., 2002). The source of protons involved in these central nervous system functions is unknown. It was speculated that protons released from the acidic synaptic vesicles along with neurotransmitters may modulate synaptic transmission (Zha et al., 2006). Given that the activity of receptors for neurotransmitters can be modified by pH (reviewed in Kaila, 1994), a role of protons as a secondary modulator of synaptic activity seems plausible.

This view, however, may change with the compelling evidence presented by Beg et al. (2008) in this issue of *Cell*. Through an elegant combination of in vitro and in vivo analyses, this study demonstrates that protons are the primary activator of target cells during a muscle contraction required for defecation in the nematode *Caenorhabditis elegans*. In this organism, defecation behavior is a rhythmic cycle that is initiated by a pacemaking posterior intestinal cell (Dal Santo et al., 1999; Peters et al., 2007) and is followed by the sequential contractions of the posterior, anterior, and rectal muscles (Croll, 1975; Thomas, 1990) (Figure 1A). Beg et al. (2008) identified two *C. elegans* mutants, *pbo-4* and *pbo-5*, where the contraction of posterior muscles is selectively abolished. Characterization of these mutants revealed that protons are released from the posterior intestinal cells through a Na^+/H^+ exchanger (PBO-4) and then activate a receptor of the Cys-loop ligand-gated ion channel

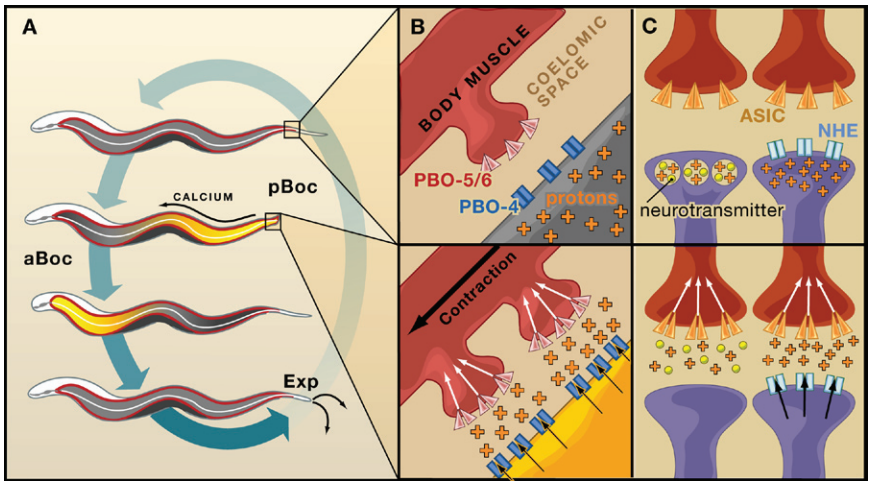

Figure 1. Protons Mediate Cell-Cell Communication
(A) The *C. elegans* defecation cycle. The 50 s rhythmic cycle consists of three motor steps: posterior body muscle contraction (pBoc), anterior body muscle contraction (aBoc), and rectal muscle contraction leading to expulsion (Exp). The cycle is initiated by a calcium ion spike generated in a posterior intestinal cell. The calcium ion spike precedes pBoc and travels anteriorly as a wave (yellow) through gap junctions between intestinal cells. This anterior calcium ion spike is required for anterior body muscle contraction and rectal muscle contraction.
(B) Initiation of pBoc by protons. Beg et al. (2008) show that proton transmitters at intestino-muscular junctions cause pBoc. The calcium ion spike, through an unknown mechanism, leads to activation of PBO-4, which releases protons into the coelomic space (bottom panel). Released protons open the PBO-5/6 ion channels, resulting in an influx of cations and contraction of posterior muscles.
(C) Protons as potential neurotransmitters. Protons are thought to modulate neurotransmission at the synapse (left). Combined with previous studies on neural acid-sensing proton-gated cation channels (ASICs), the results from Beg et al. (2008) suggest a model in which protons are neurotransmitters that activate ASICs at specific synapses in the worm (right). NHE, Na^+/H^+ exchanger.

superfamily (PBO-5) on posterior muscle cells, inducing their contraction (Figure 1B). Cys-loop channels are usually homo- or heteropentamers. The *C. elegans* genome encodes a close homolog of PBO-5, PBO-6, that may function as its coreceptor.

Muscles are usually innervated by neurons, and their contractions are stimulated by neurotransmitters. The results of Beg et al. (2008) support the intriguing model that protons are the primary inducer of muscle contractions in the worm defecation cycle. First, PBO-4 and PBO-5 proteins are localized at the appropriate places. Consistent with PBO-4's role in releasing protons into the intestino-muscular junctions or the "coelomic space," this protein is localized basolaterally in the posterior intestine. Meanwhile, PBO-5, the proton receptor, is expressed in posterior muscles. Second, protons induce posterior muscle contractions. Using a green fluorescent protein (GFP) sensor that is quenched in acidic environments, Beg et al. (2008) detect cyclic proton spikes on the basolateral surface of the intestine that precede each posterior muscle contraction. To determine if proton spikes cause muscle contractions, they injected caged protons into the coelomic space and found that the protons when uncaged induce posterior muscle contractions. Third, proton-activated posterior muscle contractions depend on PBO-4 and PBO-5. Consistent with the PBO-4 Na^+/H^+ exchanger being the proton source, PBO-4 is required for generating proton spikes, and uncaging protons is sufficient to restore posterior muscle contractions in *pbo-4* worm mutants. In contrast, proton spikes were normal in *pbo-5* mutants, but uncaging protons failed to restore muscle contractions, consistent with the notion that the PBO-5 channel is the proton receptor. This was further validated in *Xenopus* oocytes where PBO-5 elicited robust proton-gated currents when coexpressed with PBO-6, indicating that these two proteins together may act as a proton receptor.

This study reveals the presence of cyclic proton spikes in vivo and indicates that protons may serve as messengers for intercellular communications in many contexts including the nervous system. The kinetics of proton spikes indicates that the release of protons, similar to that of neurotransmitters, can be temporally and spatially regulated. Together with the elusive roles of ASICs in synaptic morphology and function (Vukicevic and Kellenberger, 2004; Wemmie et al., 2002; Zha et al., 2006), the new findings raise the intriguing possibility that protons act as neurotransmitters and induce membrane depolarization in the central nervous system (Figure 1C). It will be important to understand the mechanism of regulated proton flux. In addition, the synergy between protons and serotonin during the activation of PBO-5/PBO-6 receptors suggests that proton-gated receptors may also function together with classical neurotransmitter receptors to modulate synaptic transmission. Although the coexpression of PBO-5 and PBO-6 is required for robust induction of proton-gated currents in *Xenopus* oocytes, the loss of PBO-6 alone displays no obvious defects in defecation, suggesting that PBO-5 and PBO-6 homo- and hetero-receptors may display different physiological properties during proton activation.

The Beg et al. (2008) findings further open the door to understanding the temporal integration of a physiologically complex rhythmic cycle. The 50 s *C. elegans* defecation cycle commences as a posterior intestinal cell initiates a calcium ion spike (Figure 1A) that precedes each posterior muscle contraction (Dal Santo et al., 1999; Peters et al., 2007). A calcium ion wave propagates anteriorly and subsequently induces anterior and rectal muscle contractions (Peters et al., 2007). The execution of individual motor steps is regulated separately but is temporally integrated in the cycle (Thomas, 1990; reviewed in Branicky and Hekimi, 2006), a theme that may apply to other rhythmic cycles. It is enticing to speculate that the onset of each proton spike is initiated by a calcium ion spike in the posterior intestine that induces a posterior muscle contraction. The decay of this proton spike must be coordinated with the anterior-moving calcium ion wave so that the posterior muscles relax prior to the contraction of anterior muscles. Interestingly, the activity of mammalian Na^+/H^+ exchangers can be modulated by calcium ions through their calmodulin-binding domains and by phosphorylation mediated by a calcium-sensitive protein kinase C (Slepkov et al., 2007). The presence of a calmodulin-binding domain, protein kinase C phosphorylation sites, and a PIP_2-binding domain in the PBO-4 Na^+/H^+ exchanger supports the model of calcium-modulated proton release. Deciphering the mechanisms that regulate proton release and mediate the temporal coordination of the different motor events in *C. elegans* defecation will provide key insights into this new form of cell-cell communication, as well as the regulation of complex rhythmic cycles.

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