HHS Public Access

Author manuscript

Curr Biol. Author manuscript; available in PMC 2016 September 30.

Published in final edited form as:

Curr Biol. 2012 December 4; 22(23): R1000–R1001. doi:10.1016/j.cub.2012.10.024.

Rhythmic Ca^{2+} Signaling: Keeping Time with MicroRNAs

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Abstract

Pacemaker cells are specialized cell types that drive biological rhythms like the heartbeat and intestinal peristalsis. What determines whether a cell functions as a pacemaker? Studies in *Caenorhabditis elegans* suggest that pacemaking activity may be controlled in part by microRNAs.

MicroRNAs (miRNAs) comprise a fundamentally important new class of gene expression regulators. These tiny RNA molecules are first transcribed as long primary miRNA 'hairpin' structures from introns of protein-coding genes or from independent miRNA genes. After being processed into shorter hairpin fragments, precursor miRNAs are transported into the cytoplasm and cleaved into single-stranded 20–23 nucleotide miRNAs that associate with a ribonucleoprotein silencing complex termed RISC. A RISC-associated miRNA binds via base pairing to the 3′ UTR of target mRNAs. Binding blocks translation or triggers mRNA degradation. miRNAs may target multiple mRNA species and are predicted to control the expression of more than 60% of human protein-encoding genes, thus providing a powerful mechanism for rapid modulation and fine tuning of protein expression to match constantly changing cellular needs [1].

The list of cellular functions that miRNAs control is growing rapidly and includes regulatory roles in circadian rhythms (for example, [2,3]). A paper published in this issue of *Current Biology* by Kemp *et al.* [4] provides new insights into both miRNA biology and miRNA function in biological timekeeping. This paper demonstrates that the miRNA miR-786 regulates defecation in the roundworm *Caenorhabditis elegans*. To the uneducated biologist or non-biologist, defining the mechanisms of nematode defecation might seem to qualify for an infamous Golden Fleece Award or turn up in a political speech as an example of what's wrong with government spending. However, it's the underlying biology that's fundamentally important. In *C. elegans*, defecation is controlled by highly rhythmic intracellular Ca^{2+} oscillations in intestinal epithelial cells. Calcium signaling controls countless cellular processes ranging from fertilization to programmed cell death. The specificity of Ca^{2+} signaling is defined by the spatial localization and variation in amplitude, frequency and duration of an intracellular Ca^{2+} change [5]. The numerous experimental advantages of *C. elegans*, including forward and reverse genetic tractability, make the worm ideally suited for defining the genetic pathways underlying Ca^{2+} signaling networks and biological rhythms.

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Nematodes defecate rhythmically once every 50 seconds [6,7]. The cycle is initiated by posterior body wall muscle contraction (pBoc), which is easily observable under a simple dissecting microscope and is thus amenable to mutagenesis screening and forward genetic analysis. Using this approach, Iwasaki *et al.* [8] identified 12 genes required for normal pBoc rhythm. One of the genes, *itr-1*, encodes the *C. elegans* inositol 1,4,5-trisphosphate (IP_3) receptor [9]. IP_3 receptors mediate intracellular Ca^{2+} release from endoplasmic reticulum (ER) Ca^{2+} stores in response to changes in IP_3 and local Ca^{2+} levels [5]. Mutations in *itr-1* or overexpression of the gene disrupts pBoc timing [9].

The combined use of genetic analyses, transgenics and sophisticated cell physiological tools, including patch clamp electrophysiology and dynamic measurements of intracellular Ca^{2+} and H^+ levels, has made it possible to develop a detailed model of Ca^{2+} signaling events that drive pBoc. The *C. elegans* intestine comprises 20 epithelial cells that are connected by gap junctions and are surrounded by and in close contact with body wall muscle cells. pBoc is triggered by a transient Ca^{2+} increase in the posterior-most cells of the intestine. This Ca^{2+} increase spreads anteriorly through gap junctions into neighboring cells where it induces ER Ca^{2+} release via ITR-1 and propagation of a Ca^{2+} wave along the intestine [10,11]. Increased intestinal cell Ca^{2+} activates a basolateral Na^+/H^+ exchanger [12,13], and H^+ extrusion into the pseudocolelomic space triggers pBoc by activating proton-gated cation channels in the muscle cells [13]. Posterior intestinal cells thus function as a pacemaker to rhythmically drive Ca^{2+} waves and pBoc. Since isolated intestines show rhythmic Ca^{2+} oscillations and waves similar to those in the intact animal, this pacemaker activity is intrinsic to the posterior intestinal cells [14].

How is the timing of Ca^{2+} oscillations in the posterior intestine regulated? Kemp *et al.* [4] demonstrate that miR-786 is expressed in the two most posterior intestinal cells, is required for normal pBoc rhythm, and functions upstream of ITR-1. Loss of miR-786 function causes a lengthened and arrhythmic pBoc cycle, arrhythmic intestinal Ca^{2+} oscillations and ectopic Ca^{2+} wave initiation.

miR-786 has multiple possible gene targets. Of the 23 putative targets tested, only knockdown of *elo-2* affected the pBoc rhythm, suggesting that it functions with miR-786 to regulate pBoc timing. *elo-2* encodes a fatty acid elongase required for palmitic acid elongation. *elo-2(RNAi)* worms have elevated palmitate levels and altered fatty acid composition [15]. In miR-786 mutant worms, the lengthened and arrhythmic pBoc cycle is suppressed by culturing them on growth medium supplemented with palmitate.

A GFP reporter construct containing the *elo-2* promoter and 3′ UTR is expressed at low levels in the posterior-most intestinal pacemaker cells in about 60% of wild-type GFP transgenic worms. However, in miR-786 mutants, only about 25% of worms have reduced expression of the GFP reporter in the posterior intestine. Mutation of the putative miR-786-binding site in the *elo-2* 3′ UTR increases GFP reporter expression in the posterior intestinal cells of wild-type worms, but has no effect in miR-786 mutants. These results suggest that miR-786 functions to suppress *elo-2* expression in the posterior intestinal pacemaker cells.

In wild-type worms, *elo-2* knockdown induces the initiation of rhythmic but more frequent Ca^{2+} waves from the posterior intestine. Interestingly, even though RNAi silencing of *elo-2* suppresses arrhythmic intestinal Ca^{2+} oscillations in miR-786 mutant worms, it does not suppress ectopic Ca^{2+} wave initiation. Kemp *et al.* [4] suggest that these results are consistent with a model in which variation in *elo-2* expression along the intestine determines whether an intestinal cell functions as a pacemaker. *elo-2* expression in wild-type worms is lowest in posterior intestinal cells due to suppression by miR-786. Loss of miR-786 function leads to increased *elo-2* expression in the posterior intestinal cells. These cells thus no longer function as pacemakers and Ca^{2+} waves instead initiate ectopically.

How could low *elo-2* expression induce pacemaker activity? Calcium spikes clearly occur in cells throughout the intestine as demonstrated by ectopic Ca^{2+} wave initiation in miR-786 mutants. It is the timing of the spikes that determines the point of Ca^{2+} wave initiation. In wild-type worms, Ca^{2+} spiking is more rapid in posterior intestinal cells.

The TRPM channels GON-2 and GTL-1 are part of the Ca^{2+} signaling machinery in the intestine [16,17]. Patch clamp studies in cultured intestinal cells have demonstrated that the activity of these channels oscillates and that they are controlled by intracellular Ca^{2+} and phosphatidylinositol 4,5-bisphosphate (PIP_2) levels, and by phospholipase Cg, which hydrolyzes PIP_2 to generate IP_3 [16–18]. Calcium entry through GON-2 and GTL-1 contributes to the overall cytoplasmic Ca^{2+} increase during spiking and also likely triggers Ca^{2+} release from the ER via ITR-1. Given that GON-2 and GTL-1 are regulated by membrane lipids and the activity of lipid-bound enzymes, it is easy to envision how cell lipid composition induced by differences in *elo-2* expression could impact the timing of Ca^{2+} signaling events.

Much more work is needed to define the specific role of ELO-2 in Ca^{2+} signaling. Nevertheless, the studies of Kemp *et al.* [4] provide important new insights into two fundamental biological processes — oscillatory Ca^{2+} signaling and biological timekeeping. Their studies also raise additional intriguing questions. If miR-786 expression bestows pacemaker status on the posterior intestine, what regulates the expression of miR-786? In miR-786 mutants, what determines the site of Ca^{2+} wave initiation? Is wave initiation stochastic, or do other cellular factors also regulate cellular pacemaker activity? Do changes in fatty acid metabolism induced by diet or the presence of harmful microorganisms play a physiologically important role in regulating defecation cycle timing and the force of associated muscle contractions? As with innumerable other fundamental biological problems, *C. elegans* will teach us much more about Ca^{2+} signaling, miRNAs and biological rhythms.

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