Germ Cells Need Folate to Proliferate

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In this issue of *Developmental Cell*, Chaudhari and colleagues (2016) use a novel method to create an in vitro proliferative cell line from tumorous *C. elegans* germ cells, and in the process discover that bacterial folates act as signals for proliferation, independent of their roles as vitamins.

Metabolic pathways link nutrients to energy usage, storage, and biomass. Nutrients or metabolites within these pathways may also be sensed as signals, allowing organisms to coordinate physiological processes with the environment. In this issue of *Developmental Cell*, Chaudhari et al. find that products of bacterial folate synthesis provide a proliferative signal to *C. elegans* germline cells. Although folate metabolism has important roles in production of purines, glutathione, and s-adenosylmethioine (the donor for most methylation reactions) (reviewed in Suh et al., 2001), the authors show that bacterial folates signal to germline stem cells rather than entering the nematode folate cycle (Figure 1). Importantly, the authors made their discovery in the process of developing primary culture system from tumorous *C. elegans* germlines. Thus, Chaudhari, Kipreos and colleagues have overcome a major technical barrier in the *C. elegans* field and used this technology to uncover the communication between bacterial metabolism and nematode fertility.

Folates are B vitamins necessary in all animal cells but produced only by microbiota (Suh et al., 2001). Animals may obtain folates directly from resident bacteria in the colon or through the food chain, as other plants or animals that have taken up bacterial folates are consumed. In humans, folate is required throughout life: deficiency can cause low birth weight and neural tube defects during pregnancy, stunt growth during childhood, or cause anemia and correlate with colon cancer in adults (Suh et al., 2001). However, many of these functions derive from folate's role as an essential vitamin, and any potential role of microbial or dietary folate as a signal to cells has not been described.

In *C. elegans*, folate metabolism has been studied for its effects on lifespan and fertility and life history phenotypes. Early studies showed that folate transport was essential for normal lifespan. More recent studies focusing on interactions between folate metabolism in *E. coli* (food/microbiota) and host (*C. elegans*) metabolism have greatly expanded the significance of worms as a model system for folate metabolism, because complex effects on microbiota or host can be analyzed. For example, Virk et al. found that limiting folate in *E. coli* can affect

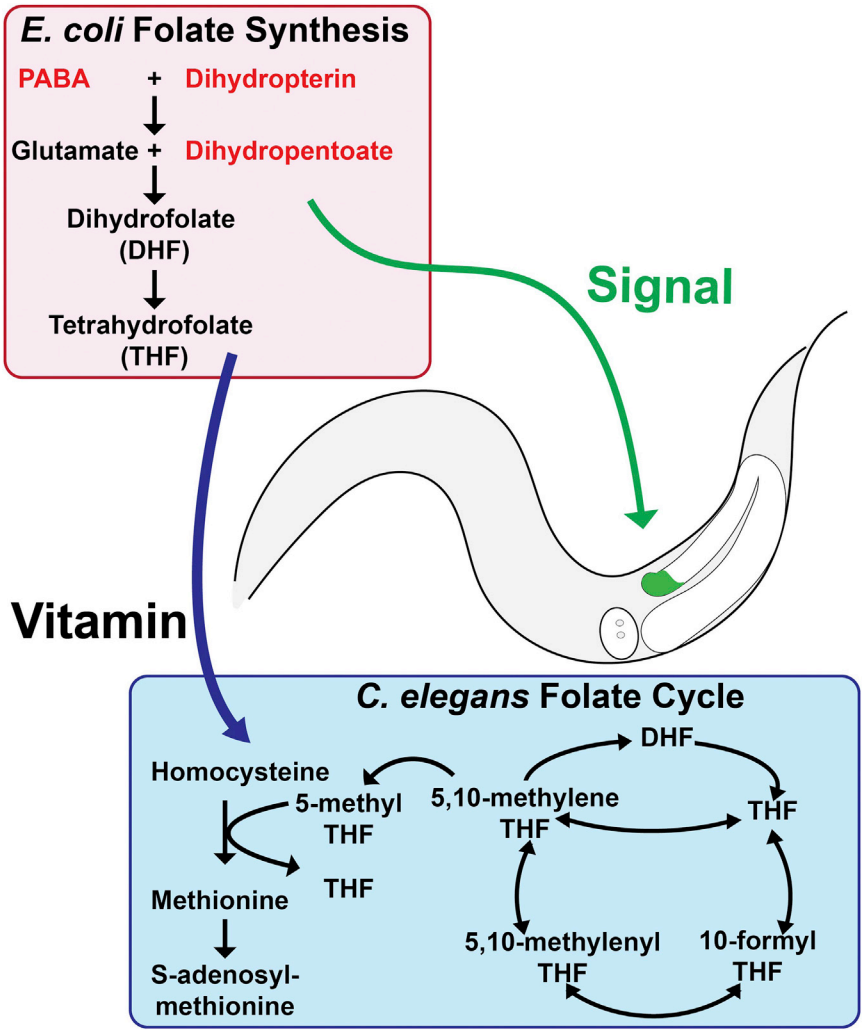

Figure 1. Bacterial Folates Can Stimulate Germ Cell Proliferation in *C. elegans*
PABA denotes para-aminobenozic acid. The *C. elegans* germline is white, with the mitotic region of germline stem cells marked in green. Metabolic diagrams adapted from Chaudhari et al. (2016).

C. elegans lifespan (Virk et al., 2016) and Cabreiro et al. found that extension of *C. elegans* lifespan by the drug metformin is mediated by effects on the folate cycle in both bacteria and worms (Cabreiro et al., 2013). Finally, the Walhout lab has demonstrated that variations in the availability of another B vitamin, B_{12}, in the worms' bacterial diet affect life history traits through interactions with the folate cycle (reviewed in Yilmaz and Walhout, 2014). These papers largely address the effects of folate cycle alteration through folate's role as a vitamin—i.e., its direct involvement in metabolic cycles. Chaudhari et al. provide a different, but not mutually exclusive, role for folates in their study as a signal for proliferation. By demonstrating that non-metabolizable bacterial folates can stimulate germ cell or seam cell divisions, the authors make an important distinction between metabolites that are sensed and ones that directly enter anabolic pathways.

It is striking that Chaudhari et al. (2016) made their initial observations while developing media for in vitro culture of proliferating *C. elegans* cells. The lack of a robust *C. elegans* cell line has been limiting, and the *C. elegans* field is rife with stories of attempts to develop a continuously proliferating line. Current methods allow for the isolation of early embryonic cells with limited division capacity (Goldstein, 1992) or mixed populations of later embryonic (Christensen et al., 2002) or larval (Zhang et al., 2011) cells suitable for single-cell assays, such as electrophysiology or library construction. However, more-uniform cell lines with less-restrictive division potential useful for biochemical or large-scale RNAi experiments, like *Drosophila* S2 cells, have been difficult to derive. Chaudhari and colleagues (2016) solve this by isolating cells from tumorous germlines. In the wild-type *C. elegans* syncytial gonad, nuclei near the distal tip undergo mitosis in response to Notch signaling and nutritional input from insulin (Hubbard, 2011); therefore, there are no individual cells for isolation. However, nuclei undergoing continuous proliferation due to combined Notch stimulation (*glp-1(gf)*), cell-cycle promotion (*cki-1*), and *daf-16*—which inhibit mitotic germ cell proliferation—are cellularized and can thus be dissociated and proliferate in culture for at least 25 generations (Chaudhari et al., 2016). While the capabilities of these cells to undergo RNAi, express transgenes, or be expanded for biochemical assays were not directly tested, it seems likely that these conditions could provide a welcome in vitro tool to complement studies in whole animals.

The folate cycle provides essential metabolic functions and connects to physiological processes in fertility, lipid accumulation, and lifespan in *C. elegans* and mammals (Suh et al., 2001; Walker et al., 2011; Yilmaz and Walhout, 2014) Many of these phenotypes may occur as metabolites from the folate/1-carbon cycle such as nucleotides, the methyl donor s-adenosylmethionine, or homocysteine become limiting or accumulate. However, we do not fully understand how cellular mechanisms, such as those driving mitosis or transcription, sense and respond to these extracellular nutrients or internal changes in metabolic flux. Chaudhari et al. (2016) have provided an intriguing example of a metabolite acting as a signal for proliferation; the next steps exploring how that metabolite interacts with mechanisms controlling cell division should be equally exciting.

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