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The *C. elegans* lethal gut-obstructed *gob-1* gene is trehalose-6-phosphate phosphatase

Jay D. Kormish [1], James D. McGhee *

Department of Biochemistry and Molecular Biology, Genes and Development Research Group, University of Calgary Faculty of Medicine, Health Sciences Centre, Room 2205, 3330 Hospital Drive, N.W., Calgary, Alberta, Canada T2N 4N1

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Abstract

We identified the *gob-1* (*g*ut-*ob*structed) gene in a forward genetic screen for intestinal defects in the nematode *Caenorhabditis elegans*. *gob-1* loss of function results in early larval lethality, at least in part because of a blocked intestinal lumen and consequent starvation. The *gob-1* gene is first expressed in the 8E cell stage of the embryonic intestine, and the GATA factor ELT-2 is sufficient but not necessary for this early phase of *gob-1* expression; *gob-1* expression later becomes widespread in embryos, larvae, and adults. GOB-1 is a member of the HAD-like hydrolase superfamily and shows a robust and specific phosphatase activity for the substrate trehalose-6-phosphate. Trehalose is a glucose disaccharide found in bacteria, fungi, plants, insects, and nematodes but not in mammals. Trehalose plays a number of critical roles such as providing flexible energy reserves and contributing to thermal and osmotic stress resistance. In budding yeast and in plants, the intermediate in trehalose synthesis, trehalose-6-phosphate, has additional critical but less well-defined roles in controlling glycolysis and carbohydrate metabolism. Strong loss-of-function mutants in the *C. elegans tps-1* and *tps-2* genes (which encode the two trehalose phosphate synthases responsible for trehalose-6-phosphate synthesis) completely suppress the lethality associated with *gob-1* loss of function. The suppression of *gob-1* lethality by ablation of TPS-1 and TPS-2, the upstream enzymes in the trehalose synthesis pathway, suggests that *gob-1* lethality results from a toxic build-up of the intermediate trehalose-6-phosphate, not from an absence of trehalose. GOB-1 is the first trehalose-6-phosphate phosphatase to be identified in nematodes and, because of its associated lethality and distinctive sequence properties, provides a new and attractive target for anti-parasitic drugs.

Keywords: *Caenorhabditis elegans*; Intestine; Trehalose; *gob-1*; Trehalose-6-phosphate phosphatase; ELT-2 GATA factor; Haloacid dehalogenase (HAD) hydrolase

Introduction

The *Caenorhabditis elegans* intestine is a straight relatively uniform tube consisting of twenty cells, all clonally derived from a single cell (the E cell) of the eight cell embryo (Sulston et al., 1983). The worm intestine is thus one of the simplest organs in multicellular animals. Many of the cellular and subcellular events that lead to intestine morphogenesis in the embryo have been described in detail (Sulston et al., 1983; Hedgecock and White, 1985; Leung et al., 1999). In addition, the transcription factor network regulating endoderm formation has been well studied, and a plausible chain of regulation can now be proposed, starting with the maternally provided SKN-1 transcription factor (Bowerman et al., 1992, 1993), proceeding through one or two waves of interim zygotic factors involved in endoderm specification (Zhu et al., 1997, 1998; Maduro and Rothman, 2002; Maduro et al., 2001; Broitman-Maduro et al., 2005; Goszczynski and McGhee, in press) and ending with transcription factors that regulate genes associated with terminal differentiation (Hawkins and McGhee, 1995; Fukushige et al., 1998; An and Blackwell, 2003; Fukushige et al., 2003). What is not known in any detail is how these two areas of study are related, i.e., how the transcription factor network initiates and maintains the detailed morphological processes that are involved in building a functioning intestine.

We have been studying the GATA factor ELT-2 (Hawkins and McGhee, 1995). ELT-2 may be the single most important intestine-specific transcription factor acting after endoderm specification (Fukushige et al., 1998, 1999; Moilanen et al., 1999; Fukushige et al., 2003, 2005), possibly regulating the

* Corresponding author. Fax: +1 403 270 0737.
E-mail address: jmcghee@ucalgary.ca (J.D. McGhee).
[1] Present address: Cell and Developmental Biology Program, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA.

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majority of genes involved in intestine differentiation. A knockout mutation of the *elt-2* gene is lethal: *elt-2* null embryos hatch but arrest as L1 stage larvae; these larvae eventually die (probably) of starvation, since the intestine lumen appears completely occluded (Fukushige et al., 1998). This *g*ut-*ob*structed (Gob) phenotype is distinctive and has been made the basis of a forward genetic screen to identify genes involved in intestinal morphogenesis, possibly direct downstream targets of ELT-2.

In the current paper, we identify one particular gene (named *gob-1*) that, when mutated, produces the gut-obstructed Gob phenotype. In the early embryo, *gob-1* is expressed only in the intestine (E) lineage, although expression later becomes widespread. As will be described below, *gob-1* appears to be a reasonable candidate for a direct downstream target of ELT-2. However, the most interesting feature of *gob-1* is quite unexpected, namely, that *gob-1* encodes the *C. elegans* enzyme trehalose-6-phosphate phosphatase. Thus, the question arises: how does loss of an enzyme involved in carbohydrate metabolism cause a lethal obstruction of the intestinal lumen? To address this question, we must provide a brief background on the many important biological features of trehalose.

Trehalose is a non-reducing glucose disaccharide that is synthesized by bacteria, fungi, insects, and nematodes but is apparently not produced by mammals (see review in Elbein et al. (2003)). Besides acting as an important energy reserve, trehalose has been demonstrated, in a number of organisms including nematodes, to play crucial roles in protecting against most environmental stresses: heat, cold, and freezing, oxidative stress, anoxia, osmotic stress, and, in the extreme, desiccation and anhydrobiosis (Behm, 1997; Elbein et al., 2003). Trehalose is widespread in nematodes (including *C. elegans*), and levels are generally in the range of 1% of dry weight (Behm, 1997). However, in species that undergo anhydrobiosis, trehalose levels have been measured up to 8–9% of dry weight. Possibly more relevant to the current study, trehalose concentrations found in nematode perivitelline fluid surrounding the developing embryo are in the range of 0.1 to 0.5 M (Behm, 1997).

In most organisms (including nematodes), trehalose is synthesized by a two-step pathway: UDP-glucose and glucose-6-phosphate are combined by the enzyme trehalose phosphate synthase to produce the intermediate molecule trehalose-6-phosphate, which is then dephosphorylated by the enzyme trehalose-6-phosphate phosphatase to produce the final product trehalose (Behm, 1997; Elbein et al., 2003). Two trehalose phosphate synthase enzymes (TPS-1 and TPS-2) have been identified in *C. elegans* (Pellerone et al., 2003), as well as in the anhydrobiotic nematode *Apelenchus avenae* (Goyal et al., 2005). Simultaneous RNAi targeting of both *tps-1* and *tps-2* in wild-type *C. elegans* results in lowering trehalose levels to ~7% of control levels; however, such trehalose depleted worms show little obvious phenotype, even under conditions of stress (Pellerone et al., 2003). Nonetheless, in the background of an *age-1* mutant, RNAi to *tps-1* and *tps-2* greatly decreases *C. elegans* resistance to osmotic shock; moreover, *age-1* worms show a twofold increase in trehalose levels even in the absence of osmotic shock (Lamitina and Strange, 2005). Furthermore, *tps-1* and *tps-2* have been identified among genes responding to *daf-2*/*daf-16* signaling and to osmotic stress, indicating an important but as yet undefined role of trehalose in aging/stress response pathways (Lamitina and Strange, 2005). Up to the present paper, trehalose-6-phosphate phosphatase, the second enzyme in the trehalose synthetic pathway, has not been identified in *C. elegans* or, to our knowledge, in any nematode (Behm, 1997; Pellerone et al., 2003; Goyal et al., 2005).

As if its roles in providing energy reserves and stress protection were not sufficient, trehalose has been shown to play critical roles in regulating glycolysis, especially in fungi and in plants (Francois and Parrou, 2001; Leyman et al., 2001; Eastmond and Graham, 2003; Eastmond et al., 2003; Gancedo and Flores, 2004). In *Saccharomyces cerevisiae*, loss of trehalose phosphate synthase function leads to an inability to metabolize glucose (and other hexoses); loss of trehalose phosphate phosphatase activity leads to temperature-sensitive growth (Gancedo and Flores, 2004). Similarly, in *Arabidopsis*, loss of trehalose phosphate synthase function is embryo lethal, and loss of trehalose phosphate phosphatase function causes thermal sensitivity (Leyman et al., 2001; Eastmond and Graham, 2003; Eastmond et al., 2003; Schluepmann et al., 2003, 2004). One theme that has emerged from this considerable body of biochemical work is that trehalose synthetic enzymes and/or trehalose-6-phosphate act as "gatekeepers of glycolysis". At least part of this control can be traced to the fact that trehalose-6-phosphate is a direct inhibitor of the *S. cerevisiae* hexokinase (Blazquez and Gancedo, 1994; Blazquez et al., 1993), but this cannot be the whole explanation (see, for example, Bonini et al. (2003)). Overall, these studies have revealed a complex network of metabolic interactions centered on trehalose, crucial for cellular homeostasis and for responses to the environment. In the current paper, we provide evidence that trehalose-centered metabolic networks are also likely to exist in *C. elegans*, with important implications for development.

Materials and methods

Strains and alleles

C. elegans strains were cultured as previously described (Brenner, 1974). The following strains and mutant alleles were used: N2 Bristol strain, Hawaiian CB4856 CB subclone of HA-8, *mnDp1* (X; V)/+; *gob-1(ca17)*, *tps-1(ok373)*, *tps-2(ok526)*, *elt-2(ca15)*.

Detection of the gut-obstructed phenotype

The gut-obstructed phenotype was detected using the following bead feeding assay. A mixture containing 40 μl of 1% fluorescent latex bead stock solution (approximately 0.19 μm in diameter, Bangs Beads, Fisher, IN), 10 μl of an overnight OP50 bacteria culture, and 50 μl of M9 buffer was spread onto a 5-cm NGM agar plate and allowed to form a bacterial lawn. Eggs were hatched on the lawn, and larvae were allowed to feed for 4 h; the larvae were then moved to a bead-free plate and allowed to feed for an additional hour.