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Original research article

CACN-1 is required in the *Caenorhabditis elegans* somatic gonad for proper oocyte development

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ABSTRACT

CACN-1/Cactin is a conserved protein identified in a genome-wide screen for genes that regulate distal tip cell migration in the nematode *Caenorhabditis elegans*. In addition to possessing distal tip cells that migrate past their correct stopping point, animals depleted of *cacn-1* are sterile. In this study, we show that CACN-1 is needed in the soma for proper germ line development and maturation. When CACN-1 is depleted, sheath cells are absent and/or abnormal. When sheath cells are absent, hermaphrodites produce sperm, but do not switch appropriately to oocyte production. When sheath cells are abnormal, some oocytes develop but are not successfully ovulated and undergo endomitotic reduplication (Emo). Our previous proteomic studies show that CACN-1 interacts with a network of splicing factors. Here, these interactors were screened using RNAi. Depletion of many of these factors led to missing or abnormal sheath cells and germ line defects, particularly absent and/or Emo oocytes. These results suggest CACN-1 is part of a protein network that influences somatic gonad development and function through alternative splicing or post-transcriptional gene regulation.

1. Introduction

Cell-cell interactions and signaling are crucial for proper development and differentiation. Germ line development, differentiation, and maturation across species is regulated through soma-germ line interactions (Lehmann, 2012; Hanna and Hennebold, 2014; Killian and Hubbard, 2005; McCarter et al., 1997; Eppig, 1991). Somatic cells provide a niche for proper germ line differentiation and development (Jemc, 2011). Defects in development of the soma and disruption of somatically expressed genes can cause reproductive defects including ovarian failure, adeno-carcinomas, granulosa tumors and infertility (Matzuk and Lamb, 2008; Singh and Schimenti, 2015; Pangas et al., 2008; Wu et al., 2007). However, the molecular mechanisms that control somatic gonadal cell specification and development are not well understood.

The reproductive system of the nematode *Caenorhabditis elegans* is an ideal model for the study of somatic gonad development and soma-germ line interactions. The *C. elegans* reproductive system consists of two symmetrical U-shaped gonad arms connected to a shared uterus. Each somatic gonad arm is enveloped by an outer basal lamina and consists of the distal tip cell (DTC), 5 pairs of gonadal sheath cells, the spermatheca, and the spermatheca-uterine valve (sp-ut) (McCarter et al., 1997; Hubbard and Greenstein, 2000; Strome, 1986). The distal mitotic germ cell pool is the source of meiotic cells, which move proximally as they undergo gametogenesis. Sperm are produced in the last stage of larval development and stored in the spermatheca. The hermaphrodites then undergo the sperm-ooycte switch and produce oocytes throughout adulthood. Oocytes progress through meiosis as they move proximally, arresting at diakinesis of meiosis I in the proximal arm. Upon receiving cues from sperm and sheath cells, the most proximal oocyte adjacent to the spermatheca (the −1 oocyte) matures, is ovulated into the spermatheca, fertilized, and finally expelled into the uterus (Hubbard and Greenstein, 2005).

Germ cells and somatic gonadal cells arise from Z2/Z3 and Z1/Z4 precursor cells respectively (Lints and Hall, 2013). These cells and their daughters are not spatially segregated until the L2/L3 molt when the gonad begins to establish organization and structure (Kimble and Hirsh, 1979). During early L3, guided by the distal tip cells (DTC), the two gonad arms begin to elongate away from the midpoint of the animal. Germ cells proliferate throughout the arm. Midway through L3 the most proximal germ cells begin to enter meiosis (Killian and Hubbard, 2005; Hansen et al., 2004; Kimble and White, 1981). The epithelial sheath cells that surround the gonad play key roles in germ line patterning during development (Killian and Hubbard, 2005).

Sheath cells originate as five lateral pairs adjacent to the proximal germ line. As the animal develops, sheath cells migrate and expand, forming a single layer of cells covering most of the

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gonad arm (Hall et al., 1999; Hirsh et al., 1976). The distal sheath cells (Sh1-2) promote germ line proliferation and exit from meiotic pachytene (Killian and Hubbard, 2005; McCarter et al., 1997), whereas the 3 pairs of proximal sheath cells (Sh 3-5) are important for oocyte maturation and for the smooth-muscle-like contractions that push oocytes into the spermatheca for fertilization (Strome, 1986; Miller et al., 2003, 2001; McCarter et al., 1999). Defects in sheath cells, including mutations in sheath cell expressed genes like *ceh-18*, a POU class homeoprotein (Rose et al., 1997), *pro-1*, a protein involved in rRNA processing (Voutev et al., 2006; Killian and Hubbard, 2004), and *mup*-2, a troponin homolog (Myers et al., 1996), or ablation of sheath cell precursor cells (Sh1-5) (Killian and Hubbard, 2005; McCarter et al., 1997) produce an array of germ line phenotypes including tumors (Killian and Hubbard, 2005; McCarter et al., 1997; Killian and Hubbard, 2004), sperm filled proximal gonad arms that lack oocytes (Killian and Hubbard, 2005) and endo-mitotically duplicating oocytes (Killian and Hubbard, 2005; McCarter et al., 1997; Rose et al., 1997; Myers et al., 1996) (the Emo phenotype (Iwasaki et al., 1996)). Even though it is clear that sheath cell development has a crucial role in proper germ line formation, little is known about the molecular mechanisms that control sheath cell specification, development and patterning in *C. elegans*. For example, the protein LIN-9 functions in an RB-related pathway, and is necessary for the development of the correct number of somatic sheath cells, however the mechanism of this regulation remains unknown (Beitel et al., 2000).

We identified CACN-1, a protein of unknown function, conserved from yeast to humans, in a screen for regulators of DTC migration (Cram et al., 2006; Tannoury et al., 2010). CACN-1 is the homolog of Cactin, which functions with the Rel/NFKB pathway to control dorsal-ventral patterning in Drosophila (Lin et al., 2000), in innate immunity in *Litopenaeus vannamei* (Zhang et al., 2014), and with TRIM39 to negatively regulate the NF_KB pathway in human cell lines (Suzuki et al., 2015). CACN-1 lacks enzymatic activity but contains a nuclear localization signal at its N-terminus, two coiled-coiled domains and a conserved C-terminus (Schultz et al., 2000; Kosugi et al., 2009). Recently, a functional role for CACN-1 has begun to emerge. Our proteomics work suggests CACN-1 is part of a novel network containing many spliceosomal components (Doherty et al., 2014). This result is consistent with the observation that both human and *Arabidopsis thaliania* (Baldwin et al., 2013) CACTIN proteins co-purify with spliceosomal proteins (Jurica et al., 2002; Bessonov et al., 2008; Ilagan et al., 2009; Ashton-Beaucage et al., 2014), and that in *Schizosaccharomyces pombe*, Cay1/cactin promotes proper splicing and protein stability of the telomeric protein Rap1 (Lorenzi et al., 2015).

The spliceosome is responsible for the removal of introns and ligation of exons (Zahler, 2012) and for alternative splicing, which allows for variety in mRNA products and is a mechanism to regulate gene expression (Wollerton et al., 2001). The spliceosome consists of over 100 different factors including small ribonuclear-protein particles (snRNPs), accessory proteins and an array of RNA binding proteins (RBPs) (Zahler, 2012; Chen and Cheng, 2012). In the *C. elegans* genome 2562 annotated genes, or 13% of the genome, are alternatively spliced (Zahler, 2005). In addition, regulation of gene expression in the *C. elegans* germ line is primarily translational, most commonly through binding of RBPs to the 3′ UTR of target messages (Merritt et al., 2008). Even though splicing and post-transcriptional gene regulation play an important role in *C. elegans* biology, many *C. elegans* splicing factors and RBPs remain uncharacterized.

CACN-1 has been previously shown to cause germ line over-proliferation and issues in germ cell differentiation when depleted solely in the germ line of *C. elegans* (Kerins et al., 2010). However, CACN-1's role in the somatic gonad and the mechanism by which it exerts these germ line effects remains largely unknown. In this study, we demonstrate that CACN-1 is necessary primarily in the soma for the presence and proper morphology of the gonadal sheath cells that regulate the sperm-oocyte switch as well as germ line maturation and ovulation in *C. elegans*. Depletion of a set of previously identified CACN-1 interacting proteins (Doherty et al., 2014) similarly results in sheath cell and germ line defects. The results of this study reveal that CACN-1 and its network of interacting proteins are important for proper development of the gonad and suggest that CACN-1 may be involved in pre-mRNA splicing or post-transcriptional regulation to mediate these decisions.

2. Materials and methods

2.1. Nematode strains

Nematodes were grown on nematode growth media (NGM) (0.107 M NaCl, 0.25% wt/vol Peptone (Fischer Science Education), 1.7% wt/vol BD Bacto-Agar (Fisher Scientific), 0.5% Nyastatin (Sigma), 0.1 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.5% wt/vol Cholesterol, 2.5 mM KPO_4), seeded with *Escherichia coli* OP50 using standard techniques (Myers et al., 1996). Nematodes were cultured at 23°C unless specified otherwise. The strains used in this study are as follows: N2 (wild type reference strain from Bristol), NL2550 *ppw-1(pk2505)* and NL2098 *rrf-1(pk1417)* and the GFP expression lines DG1575 *lim-7::GFP* and OD27 *AIR-2::GFP*.

2.2. RNA interference

Starved dauer nematodes were allowed to recover for 48 h on NGM plates newly seeded with OP50. This procedure produces young gravid adults for egg collection. Eggs were released using an alkaline hypochlorite solution as described in Hope (1999), and washed 3 × with filter sterilized M9 buffer (22 mM KH_2PO_4, 42 mM $NaHPO_4$, 86 mM NaCl, and 1 mM $MgSO_4$) ('egg prep'). Clean eggs were then transferred to NGM previously seeded with HT115(DE3) bacteria that express dsRNA for RNAi. Strains utilized in each RNAi experiment are indicated.

The RNAi feeding protocol was performed essentially as described in Timmons et al. (2001). HT115(DE3) bacteria transformed with the dsRNA construct of interest were grown over night at 37°C in Luria broth (LB) supplemented with 40 μg/ml ampicillin. The following day, 150 μl of the culture was seeded on NGM agar supplemented with 25 μg/ml carbenicillin and isopropylthio-β-galactoside (IPTG) and incubated at room temperature for 24–72 h to induce dsRNA expression. Eggs collected from the alkaline lysis procedure were transferred onto these plates and incubated at 23°C for the times specified.

The *cacn-1* ORF RNAi clone is a full-length cDNA matching Wormbase (WS2000) predictions (Open biosystems; Huntsville, AL, USA). All CACN-1 interactor RNAi clones are described in Doherty et al. (2014). Empty pPD129.36 vector ("empty RNAi") was used as a negative control in all RNAi experiments.

2.3. RNA in situ hybridization

RNA *in situ* hybridization was performed in accordance with Lee and Schedl (2006). Nematodes subjected to both *cacn-1* and control *empty* RNAi were collected at adulthood (54 h post 'egg prep'). Gonad arms were extracted in 1 × Phosphate Buffered Saline (PBS) and fixed in 3% paraformaldehyde/0.25% glutaraldehyde/0.1 M K_2HPO_4 (pH 7.2). Samples were washed with in 1 × Phosphate Buffered Saline and 0.1% Tween 20 (PBST) and stored in cold methanol overnight, then washed with PBST and digested with Proteinase K, 50 μg/ml in PBST, for 30 min. Gonad