Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 308 (2007) 196–205
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Membrane localization of the NlpC/P60 family protein EGL-26 correlates with regulation of vulval cell morphogenesis in *Caenorhabditis elegans*

Kathleen A. Estes, Rasika Kalamegham [1], Wendy Hanna-Rose *

Department of Biochemistry and Molecular Biology, 104D Life Science Building, The Pennsylvania State University, University Park, PA 16802, USA

Received for publication 11 December 2006; revised 26 April 2007; accepted 18 May 2007
Available online 25 May 2007

Abstract

Vulval morphogenesis in *Caenorhabditis elegans* generates a stack of toroidal cells enclosing a tubular lumen. Mutation of *egl-26* is associated with malformation of vulF, the most dorsal toroid in the stack, resulting in a blocked lumen and an egg-laying defect. Here we present evidence that vulF retains the expected gene expression pattern, functions in signaling to the uterus and retains proper polarity when *egl-26* is mutated, all suggesting that mutation of *egl-26* specifically results in aberrant morphogenesis as opposed to abnormal fate specification. Recent computational analysis indicates that EGL-26, which was previously characterized as novel, belongs to the LRAT (lecithin retinol acyltransferase) subfamily of the NlpC/P60 superfamily of catalytic proteins. Via site-directed mutagenesis, we demonstrate a requirement of the putative catalytic residues for EGL-26 function *in vivo*. We also show that mutation of conserved serine 275 perturbs the apical membrane localization and the function of the EGL-26 protein. Additional mutagenesis of this residue suggests that EGL-26 attains its membrane localization via a mechanism distinct from that of LRAT.

Keywords: LRAT; Hrasls3; Morphogenesis; Organogenesis; Tubulogenesis; Palmitoyltransferase

Introduction

Formation of internal lumens is vital to the function of many organ systems, and vulval morphogenesis in *Caenorhabditis elegans* is an excellent tractable model for elucidating the molecular mechanisms controlling one type of tubular organogenesis. Vulva morphogenesis is initiated upon production of an EGF-like signal by the gonadal anchor cell (AC) (Kimble, 1981; Sulston and White, 1980). In response to this signal, three ventral hypodermal cells undergo a series of divisions. Their twenty-two progeny then complete a complex series of cell shape changes, cell migrations and cell fusions (Sharma-Kishore et al., 1999), resulting in the formation of a stack of mostly multinucleate toroidal cells surrounding a central lumen. This vulval lumen provides a pathway between the uterus and the outside of the body and is required for mating and egg-laying. During vulval morphogenesis, the AC sits atop of the developing vulval lumen and occupies the space that will become the lumen in the most dorsal toroidal cell, which is called vulF (Sharma-Kishore et al., 1999; Sherwood and Sternberg, 2003). The AC subsequently fuses to the uterine seam cell leaving only the thin uterine seam cell cytoplasm between the vulval and uterine lumens (Newman et al., 1996). This thin laminar process is presumably broken when egg-laying begins, creating an uninterrupted passageway from the uterus to the environment.

The *egl-26* gene was first identified in a screen for mutants with egg-laying (Egl) defects (Trent et al., 1983). New alleles were found in a screen for Egl mutants associated with specific vulval morphology defects (Hanna-Rose and Han, 2002). In *egl-26* mutants, the vulF cell adopts a “closed” morphology with no inner lumen as opposed to the expected toroidal morphology. This results in a blockage between the vulval and uterine lumens, a connection of gonad (Cog) defect.

To better understand the role of *egl-26* in the process of morphogenesis, we have extensively examined this novel

* Corresponding author.
E-mail address: wxh21@psu.edu (W. Hanna-Rose).
[1] Current address: Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

0012-1606/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2007.05.020

mutant phenotype. We previously hypothesized that *egl-26* acts specifically during morphogenesis rather than during specification of vulF fate (Hanna-Rose and Han, 2002). We will present data to support this model. We also demonstrate that mutation of *egl-26* does not perturb polarization of vulval cells or penetration of vulF by the AC, suggesting that the morphology defect manifests quite late in vulva development.

EGL-26 shares sequence motifs with the NlpC/P60 superfamily of enzymes (Anantharaman and Aravind, 2003). This family includes proteins that range in biochemical function from cell wall peptidases in prokaryotes to palmitoyltransferases in mammals (Anantharaman and Aravind, 2003). EGL-26 belongs to a subfamily that includes the mammalian proteins LRAT (lecithin retinol acyltransferase) and Hrasls3 (HRAS-like suppressor 3) as well as the picornovirus 2A proteins (Anantharaman and Aravind, 2003; Hughes and Stanway, 2000). The mammalian protein most closely related to EGL-26 is LRAT, which is a biochemically well-characterized palmitoyltransferase (Anantharaman and Aravind, 2003; Rando, 2002; Xue et al., 2004). LRAT palmitoylates all-*trans*-retinol (vitamin A), producing all-*trans*-retinyl esters necessary for rhodopsin chromophore production in the retinal pigment epithelium (Barry et al., 1989; MacDonald and Ong, 1988; Saari and Bredberg, 1989; Shi et al., 1993). LRAT also catalyzes the palmitoylation of RPE65 (retinal pigment epithelial protein of 65 kDa), regulating chromophore synthesis during the light–dark cycle (Xue et al., 2004). Mutation of LRAT is associated with an early-onset retinal dystrophy (Thompson et al., 2001). Much less is known about Hrasls3, which was identified in a screen for tumor suppressors in H-ras-transformed cell lines (Sers et al., 1997). Because mutation of *egl-26* results in such a specific and easily studied phenotype, the *C. elegans* vulva provides an excellent model for studying biological roles of the NlpC/P60 family in animal development.

The defining motifs of this family are the H-box domain and the NC domain (Fig. 1) (Anantharaman and Aravind, 2003; Hughes and Stanway, 2000). The H-box contains a conserved histidine, and the NC domain contains a conserved cysteine (Hughes and Stanway, 2000). These residues are essential for catalytic function of this family of enzymes (Anantharaman and Aravind, 2003; Xue et al., 2004), and we present evidence that these residues are essential for the developmental role of EGL-26 *in vivo*.

Enzymes in the NlpC/P60 superfamily are frequently associated with or function at the membrane (Anantharaman and Aravind, 2003; Hughes and Stanway, 2000). For example, a predicted C-terminal transmembrane domain of LRAT spans the membrane, targets the protein to the ER and is required for acyltransferase function in COS-7 cells (Moise et al., 2007). Interestingly, although ELG-26 does not contain a predicted transmembrane domain, EGL-26::GFP expression is highly concentrated at the apical membrane (e.g., Fig. 5B) (Hanna-Rose and Han, 2002). We present evidence correlating EGL-26 membrane localization with function during development.

Materials and methods

Maintenance and culture of C. elegans strains

Unless otherwise stated, strains were grown under standard conditions at 20° (Brenner, 1974). We used the following strains, alleles and transgenes: N2 wild-type; PS4308 *syIs107*[*unc-119*(+)+*lin-3*(*delta-pes-10*)::GFP]; MH1371 *kuIs38* [*dpy-20*(+)+*cdh-3*::GFP]; LG II: *egl-26*(*ku211*, *ku228*, *n481*) and *egl-26* (*tm1244*) (obtained from the National Bioresource Project, Tokyo Women's Medical University, Japan), MT681 *nDf3/lin-31(n301) bli-2(e768)* (Greenwald and Horvitz, 1980), BL5715 *inIs179*[*ida-1*::GFP] (Zahn et al., 2001); CB5584 *mIs12*[*myo-2*::GFP, *pes-10*::GFP, *F22B7.9*::GFP]; LG III: *unc-119(ed3)*; LG IV: PS3239 *dpy-20(e1282) syIs49*[dpy-20(+)+*zmp-1*::GFP)] (Inoue et al., 2002); LG V: *him-5(e1490)*. Additional genetic information is available at http://www.wormbase.org.

Cloning and genetics

To create vectors encoding EGL-26 mutant proteins, we performed site-directed mutagenesis of pWH15[EGL-26::GFP] (Hanna-Rose and Han, 2002) by recombinant PCR. We used primers at the site of mutagenesis (Table 1) and the following outside primers:

for H166 and H178 recombinant PCR products:
*Pfl*M I F TTCGATGATCCACCAATTGG
Sac I R CAAAATTTGCCGAGCTCGGC
for C261 and S275F recombinant PCR products:
Sac I F GTCGTCGACGAGCTCGGCAAATTTTGAGATTTACC
*Bam*H I R CGGGATCCCGAAGAAGTACTGCTGCTCGC
for S275E, S275A and S275T recombinant PCR products:
COG-4 5′ SEQ GTCTCGTGACCTCATCAGCC
GFP 3′ past Nco1 GTAGTGACAAGTGTTGGC

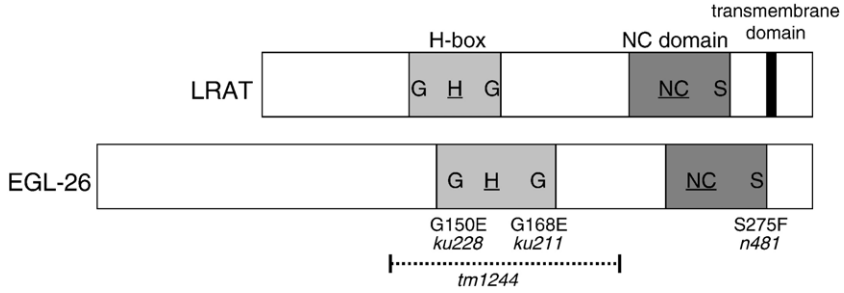

Fig. 1. Schematic comparing protein motifs between EGL-26 and LRAT. The positions of the conserved H-box (light grey boxes) and NC domains (dark grey boxes) that characterize eukaryotic NlpC/P60 superfamily proteins are indicated. The defining histidine (H) and asparagine/cysteine (NC) are underlined in the H-box and NC domain, respectively. The thick black line represents the LRAT transmembrane domain. EMS induced alleles of *egl-26* (*ku228*, *ku211* and *n481*) and the substitution mutations that they cause are indicated beneath the conserved amino acids that they alter. The line below the EGL-26 sequence represents the position of the out-of-frame deletion in the *tm1244* allele, which is predicted to encode a truncated protein that includes residues 1 to 129 plus a leucine prior to the stop codon introduced by the frame shift. Sequence identity/similarity between EGL-26 and LRAT is 34/69% in the H-box and is 38/59% in the NC domain.