Supplemental Data

FOS-1 Promotes Basement-Membrane Removal during Anchor-Cell Invasion in *C. elegans*

David R. Sherwood, James A. Butler, James M. Kramer, and Paul W. Sternberg

Molecular Methods

The 5' end of the *fos-1b* transcript was identified by the isolation of the partial cDNA yk531C4.5 from Yuji Kohara. RT-PCR analysis from wild-type mixed stage RNA identified the complete *fos-1b* cDNA. Multiple cDNA clones were sequenced to confirm the predicted protein structure. Comparison of the *fos-1* gene sequences from *C. elegans* and *C. briggsae* using the FamilyRelations program (Brown et al., 2002, Dev. Biol. *246*, 86-102) and the NetGene2 program (version 2.4, http://www.cbs.dtu.dk/services/NetGene2/) predicted the existence of the 5' start exon of the *fos-1a* transcript, which was confirmed by RT-PCR analysis. Additional 5' analysis indicated that *fos-1b* contains the *trans*-spliced leader sequence SL1 (Krause and Hirsh, 1987), while *fos-1a* does not.

The *ar105* mutation was identified by sequencing both strands of the predicted exons and splice junctions of the *fos-1* gene from PCR fragments amplified using *fos-1(ar105)* genomic DNA.

Isolation of the *zmp-1* Deletion Allele

The *zmp-1* Tc1 transposon insertion allele (*pk205*) was isolated as described (Zwaal et. al., 1993, Proc. Natl. Acad. Sci. *90*, 7431-7435; Plasterk, 1995, Methods Cell Biol. *48*, 59-80), from cultures of strain MT3126 *mut-2(r459) I; dpy-19(e12590* III using nested primers located in exon VII (B1: TGACTCGAGAAGTGAATCTGCCATTGAGAG; B2: GCTTCTCATTGCGAGGGCAGAATC) and Tc1-specific nested primers (Plasterk, 1995, Methods Cell Biol. *48*, 59-80). Strain CH1315, which contains the Tc1 excision deletion allele *cg115*, was isolated from NL741 as described (Zwaal et. al., 1993, Proc. Natl. Acad. Sci. *90*, 7431-7435; Plasterk, 1995, Methods Cell Biol. *48*, 59-80), using primers DSP3 (CTCGGTCACGTCTCTGTGCG) and M43 (TTATCCTCGAGCCGAAAGTCGAAGAGGTGC). *cg115* was out-crossed six times prior to further analysis. The breakpoints of *cg115* were determined by sequencing the product of PCR amplification of CH1315 DNA with DSP4 (AATTAGTTGACGAGACAAGTCAGG) and B3 (AGTGAAGGCAGAATGTACTCC) primers. The deletion is 2366 bp (nucleotides 965-3330 U41266) and removes the protease active site.

Gene Expression Constructs and Transgenic Animals Generated

Fos and hemicentin reporter constructs were injected into the germline (Mello et al., 1995, *Caenorhabditis elegans:* Modern Biological Analysis of an Organism, H. F. Epstein and D. C. Shakes, eds., San Diego, CA: Academic Press, pp. 452-482.

) at 50-100 µg/ml to create transgenic lines using pDP#MM016B (*unc-119*(+)) as the co-injection marker in an *unc-119*(*ed4*) background. Stable lines were then integrated (*Is*), backcrossed and mapped or maintained as extrachromosomal arrays (*Ex*)as follows: *syIs118*(pDRS46/*fos-1a*::YFP-TX)*I*, *syIs137*(pDRS61/*fos-1b*::CFP-TX)*III*, *syIs129*(phSGΔS1/hemicentin-ΔSP::GFP, Vogel and Hedgecock, 2001)*III*, *syIs123*(pDRS47/*fos-1a*::YFP-TL)*X*, *syEx683*(pDRS62/*fos-1b*::CFP-TL), *syEx684*(pDRS53/*fos-1a/b*::GFP-TL). AC-specific expression constructs pDRS63(*pAC-fos-1b*::CFP) and pDRS64(*pAC-fos-1a*::YFP) were generated using the 1479 bp mk62-63 *cdh-3* regulatory region (Kirouac and Sternberg, 2003). These constructs were injected at 150 µg/ml with the co-injection plasmid marker pPD132.102 (*myo-2*::YFP) to create the transgenic lines *syEx677*(*pAC-fos-1a*::YFP) and *syEx678*(*pAC-fos-1b*::CFP). The heat-shock construct pDRS74(*hsp16-2-fos-1a*::YFP) was injected at 50 µg/ml with pPD132.102 to create the line *syEx688*. The *cdh-3*::YFP-TL construct was created by inserting YFP at the C-terminus, and the *zmp-1*-FLAG translational fusion was constructed by inserting a FLAG tag (DYKDDDDK) immediately after the predicted furin cleavage site using a PCR fusion-based approach (Hobert, 2002, Biotechniques 32, 728-730). These fusions were injected at 0.02 µg/ml (*cdh-3*-YFP-TL) and 10 µg/ml (*zmp-1*-FLAG) with pDP#MM016B into *unc-119 (ed4)* animals to create *syEx707* and *syEx708*, respectively.

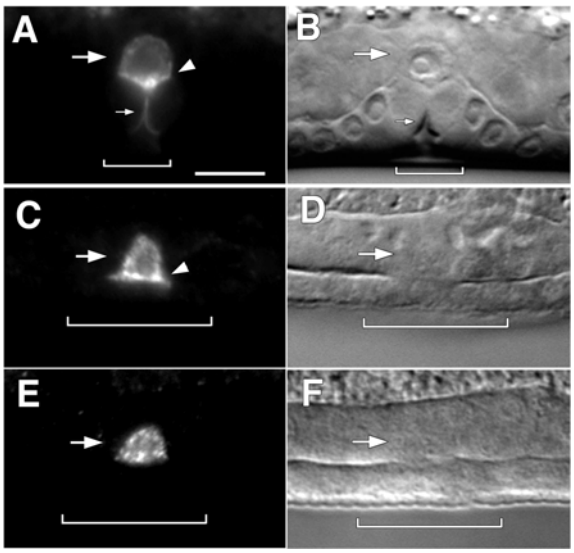
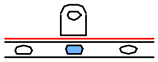
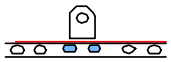
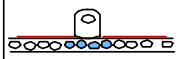
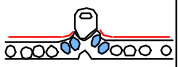
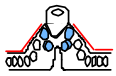
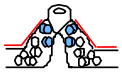


Figure S1. CDH-3 and ZMP-1 Localization in the AC during Invasion
Fluorescence (A) and corresponding Nomarski (B) image shows that CDH-3::YFP-TL localizes strongly to the invasive basolateral membrane (arrowhead) of the AC (arrow). CDH-3::YFP-TL also localizes to the lumen surface of the invaginating vulval cells (small arrow). Fluorescence (C,E) and corresponding Nomarski images (D,F) show the localization of ZMP-1-FLAG in the AC (arrows) at the time of AC invasion. ZMP-1-FLAG localizes to various sized puncta on the cell surface that often are concentrated at the invasive basolateral membrane (arrowhead, C). The nuclei are not visible in the

ZMP-1-FLAG Nomarski images because fixation alters their optical properties. The scale bar is 10 µm.

Table S1. Timing of AC Invasion, Gonad Arm Reflection, and VU Division in Wild-Type Animals

Time (h)[a]	P6.p/ larval Stage	Stage of AC invasion[b]	Gonad reflection[c]			VU descendants on each side of AC[d]			
						3/3	6/6	12/12	18/18
28	1-cell (L3)		22 (100%)	0	0	22 (100%)	0	0	0
30	2-cell (mid L3)		28 (93%)	2 (7%)	0	18[e] (60%)	12[f] (40%)	0	0
31.5	4-cell (late L3)		0	30 (100%)	0	0	30 (100%)	0	0
32.5	4-cell (late L3/ L3 molt)		0	10 (40%)	15 (60%)	0	20 (80%)	5 (20%)	0
33.5	6-cell (L3 molt/ early L4)		0	0	23 (100%)	0	1 (4%)	22 (96%)	0
35	8-cell (early L4)		0	0	23 (100%)	0	0	5 (22%)	18 (78%)

[a] Time refers to hours post-hatching at 20°C, which was inferred from the time of the L2 molt in staged hermaphrodites.

[b] Time of AC penetration through the juxtaposed gonadal and ventral epidermal basement membranes (red) and invasion into P6.p cell descendants(blue nuclei; as determined in Sherwood and Sternberg 2003).

[c] The number and (percentage) of animals displaying shown stages of gonad arm reflection (posterior arm viewed) was scored in relation to time post-hatching and AC invasion. Notably there was a strong correlation between dorsal reflection of the gonad and the time of AC invasion (approximately 31 hours post-hatching).

[d] The number and (percentage) of animals with shown ventral uterine (VU) cell divisions were scored in relation to time post-hatching and AC invasion. Animals were scored from approximately one hour after the first VU division in the early-to-mid L3 through the early L4 stage.

[e] No animals at this stage of VU development had ACs that initiated invasion through the basement membrane.

[f] Of the 12 animals viewed at this VU stage, 5 initiated invasion.