Genomes & Developmental Control

C. elegans TCF protein, POP-1, converts from repressor to activator as a result of Wnt-induced lowering of nuclear levels

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Abstract

Canonical Wnt signaling converts the TCF/LEF transcription factor from repressor to activator by increasing nuclear levels of its coactivator, β-catenin. A striking exception had been reported for Wnt-induced endoderm formation during *C. elegans* embryogenesis. It has long been believed that transcriptional activation of Wnt target genes in the endoderm precursor occurred due to a lowering of nuclear levels of the worm TCF/LEF protein, POP-1, effectively alleviating POP-1 repressive activity. Contrary to this model, we demonstrate here that POP-1 directly activates Wnt target genes in the endoderm precursor. Wnt converts POP-1 from a repressor to an activator, and this conversion requires that POP-1 nuclear levels be lowered in the endoderm precursor. We propose that the balance between TCF/LEF and coactivator(s), achieved by elevating coactivator levels (the canonical pathway) and/or reducing TCF/LEF levels (worm endoderm), determines Wnt signal strength.
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Keywords: Wnt; POP-1; Endoderm; *C. elegans*; TCF/LEF; β-catenin

Introduction

At the eight-cell stage of *C. elegans* embryogenesis, the developmental potential of one blastomere, termed the endoderm founder, E, is restricted to the entire endoderm lineage (intestine) of the worm. The specification of E for endoderm fate requires Wnt signaling (Rocheleau et al., 1997; Thorpe et al., 1997). At the four-cell stage, the EMS blastomere is polarized as a result of receiving a Wnt signal from its posterior neighbor P2, such that E, the posterior daughter of EMS, generates endoderm, whereas MS, the anterior daughter, generates mesodermal tissues (Rocheleau et al., 1997; Thorpe et al., 1997). In the absence of this P2 Wnt signal, E adopts the fate of MS and develops mesodermal tissues. In the canonical Wnt pathway, well established in mice, frogs, flies and worms, Wnt signal results in stabilization of β-catenin, its translocation to the

nucleus and interaction with an N-terminal domain of the TCF/LEF transcription factor, resulting in activation of Wnt target genes (Bienz, 1998; Brantjes et al., 2002). In the absence of Wnt signal, TCF/LEF functions as a transcriptional repressor through its interaction with the corepressor Groucho.

Endoderm induction in *C. elegans* appears to deviate in several significant ways from the canonical pathway. First, although the worm TCF/LEF homolog POP-1 is required for the fate difference between MS and E, it is required for the MS-derived cell fates rather than the E-derived, Wnt-dependent cell fate (Lin et al., 1995). In a wild-type embryo, the nuclear POP-1 level is higher in MS than in E (Lin et al., 1995, 1998). In a *pop-1* mutant, the MS blastomere adopts an E-like fate, producing excess endoderm. This is contrary to the observation in canonical Wnt signaling systems that *tcf* and Wnt mutants have similar phenotypes (Brunner et al., 1997; Molenaar et al., 1996; van de Wetering et al., 1997). Second, POP-1 appeared to function primarily as a repressor of a Wnt-responsive endoderm-determining gene, *end-1* (Calvo et al., 2001). Wnt signaling leads to a lowering

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of POP-1 nuclear levels in E, and it is believed that this results in *end-1* expression and the specification of an endodermal fate through the alleviation of POP-1 repressive activity (Calvo et al., 2001; Lo et al., 2004; Rocheleau et al., 1997; Thorpe et al., 1997). Third, the role of β-catenin in this process was unclear. Three β-catenins have been identified in the *C. elegans* genome—WRM-1, BAR-1 and HMP-2 (Costa et al., 1998; Eisenmann et al., 1998; Rocheleau et al., 1997). Of these three candidate β-catenins, only one, WRM-1, has been shown genetically to be required for specification of the endoderm precursor (Rocheleau et al., 1997). However, despite considerable effort, the accumulated evidence does not support a stable physical interaction between POP-1 and WRM-1 (Korswagen et al., 2000; Natarajan et al., 2001; Rocheleau et al., 1999). In fact, WRM-1 has been shown to play a key role in the reduction in POP-1 nuclear levels in E following Wnt signal, apparently by activation of the MAP kinase LIT-1 (Lo et al., 2004; Rocheleau et al., 1999). LIT-1 phosphorylated POP-1 is actively exported by PAR-5, a 14-3-3 protein, from the E nucleus in response to Wnt signal (Lo et al., 2004). BAR-1 can interact with an N-terminal domain of POP-1 and functions as a coactivator in later embryological processes that utilize the canonical Wnt pathway (Korswagen et al., 2000; Natarajan et al., 2001), but there is no evidence supporting a role for either *bar-1* or *hmp-2* in endoderm formation.

We report here that, contrary to popularly held belief, the expression of Wnt-responsive genes in E is not simply due to the alleviation of POP-1 repression. Instead, their expression requires direct activation by POP-1, and the Wnt-induced reduction of POP-1 nuclear levels in E is required for POP-1 to function as a transcriptional activator of Wnt target genes. The Wnt signal converts POP-1 from a repressor of endoderm-specific genes in MS to an activator of these same genes in E. Activation of the endoderm-specific genes requires the N-terminal domain of POP-1, implying that a β-catenin is involved. We discuss how these results impact the current view of canonical Wnt signaling and propose a modified canonical pathway that incorporates these data.

Materials and methods

Strains

N2 was used as the wild-type strain. Genetic markers used in this study are: LGI, *pop-1(zu189)*, *dpy-5(e61)*, *hT1(I;V)*, TX585(teIs18) and TX691(teIs46) were generated by microparticle bombardment (Praitis et al., 2001) in which $P_{sdz-23}gfp::H2B$ and $P_{end-1}gfp::H2B$ reporters, respectively, were stably integrated into the genome. All other transgenes were generated by injection and were not integrated. *wrm-1* RNAi was performed by feeding as described (Lo et al., 2004).

Plasmids

All expression clones used in this study were generated using Gateway technology (Invitrogen) (Lo et al., 2004; Robertson et al., 2004). Wild-type or mutant POP-1 was fused with GFP, CFP or YFP at the N-terminus. Reporter constructs for *sdz-23* and *sdz-26* were transcriptional fusions that drive the expression of GFP::H2B (Robertson et al., 2004), whereas that for *end-1* was a translational fusion containing a 2.2 kb *end-1* promoter, a 1.7 kb genomic sequence downstream of the AUG and GFP::H2B.

Imaging

Imaging of immunofluorescence and live embryos was performed as described (Rogers et al., 2002) except that all were taken as 16-bit images. The filter wheels (Ludl Electronic Product) and shutter controller were driven by a custom software package (*os4d 1.0*, freely available on request to jwaddle@mail.smu.edu). The experiment shown in Fig. 3 was performed with either CFP::POP-1 or YFP::POP-1. The folding of CFP is too slow for the rapid division cycles observed during early *C. elegans* embryogenesis, and therefore only very low CFP signals were observed with all fusions constructed. The separation of YFP::POP-1 and GFP::H2B was performed using a LSM 510 Meta confocal microscope (Zeiss). Similar results were obtained when either CFP or YFP was fused to POP-1. GFP quantifications were performed as in Lo et al. (2004).

Results and discussion

POP-1 is required for the repression of E-specific genes in the MS lineage

Utilizing total cDNAs prepared from individual stage-specific worm embryos of either wild-type or mutant genotype, we identified a number of genes that are newly transcribed during the approximately 30 min between the four-cell and twelve-cell embryo stages (Robertson et al., 2004). Among these genes are several expressed specifically in the MS, E or EMS lineages. We focus here on two of these genes, *sdz-23* and *sdz-26*, as promoter fusion to a GFP::histone H2B reporter gene indicated that these two genes are expressed exclusively in the E lineage (Fig. 1) (Robertson et al., 2004). A detailed characterization of these genes will follow, but for the purpose of this study, they serve simply as E lineage-specific markers. Depletion of POP-1 results in the derepression of *sdz-23* and *sdz-26* in the MS blastomere (Fig. 1). This effect is similar to that seen with the only other two previously identified E-specific genes, *end-1* and *end-3* (Figs. 1k−n and data not shown), and confirms that POP-1 represses E-specific genes, directly or indirectly, in the MS blastomere (Calvo et al., 2001; Maduro and Rothman, 2002; Zhu et al., 1997).
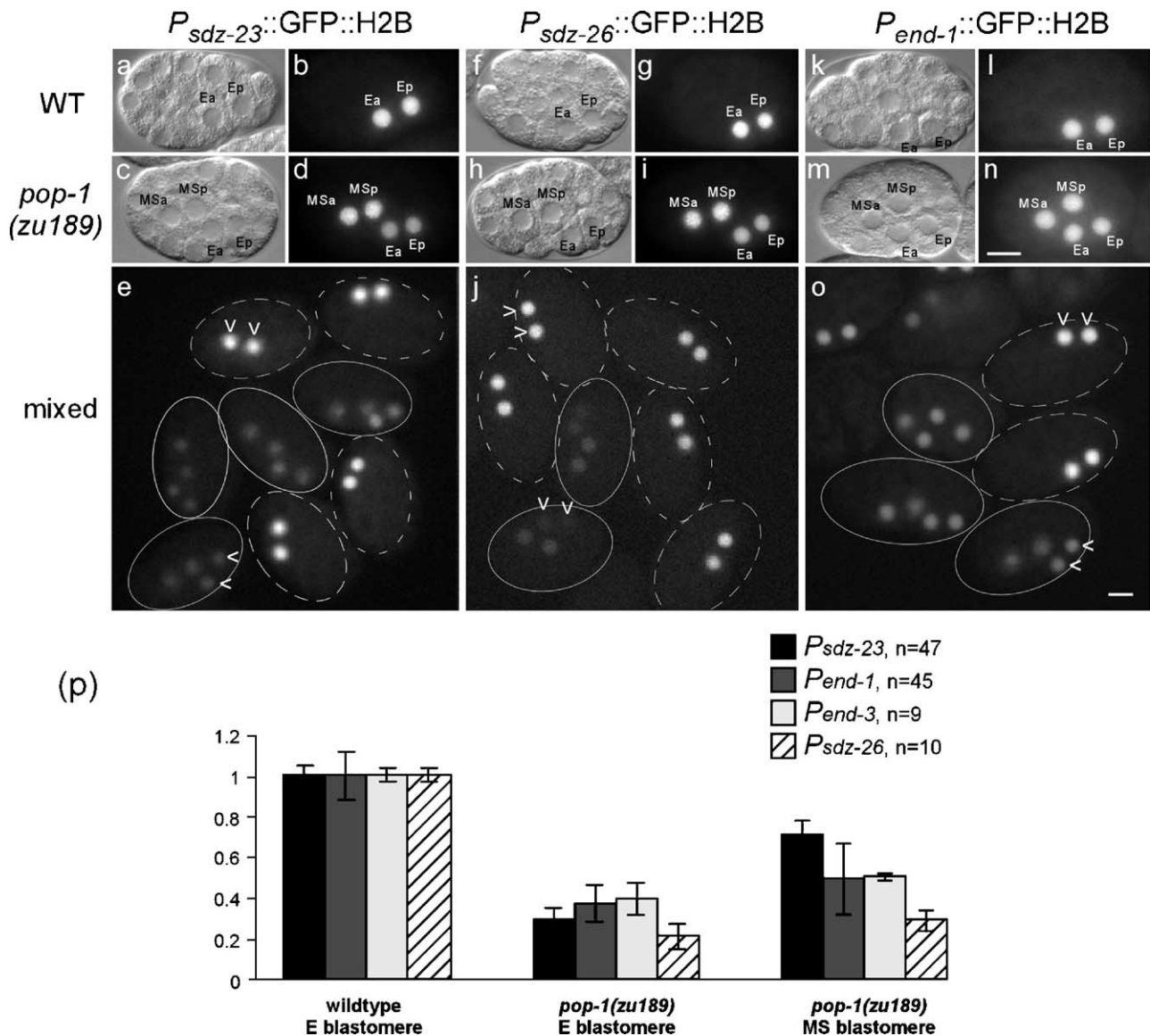
Fig. 1. POP-1 activates E-specific genes in the E blastomere and represses them in the MS blastomere. Promoter::gfp::H2B reporter constructs for sdz-23 (a–e), sdz-26 (f–j) and end-1 (k–o) expressed in either the wild-type (a, b, f, g, k, l) or the pop-1 (zu189) mutant (c, d, h, i, m, n) backgrounds. All embryos are shown at the stage when two MS (MSa and MSp) and two E (Ea and Ep) daughters are present and with the DIC image to the left and the corresponding nuclear GFP::H2B fluorescence image to the right. All three genes are derepressed in MS in pop-1(zu189) mutant embryos. (e, j, o) Wild-type (dashed outline) and pop-1 mutant embryos (solid outline) expressing GFP reporters photographed together. Open arrowheads denote Ea and Ep in one wild-type and one pop-1 embryo per field. Note that E lineage expression in the pop-1 mutant background is significantly less than in the wild-type background. (p) Quantification of GFP signals in wild-type E versus pop-1(zu189) E and MS nuclei. All quantifications were performed at the 2MS+2E stage. n: numbers of wild-type E, pop-1(zu189) MS and pop-1(zu189) E nuclei, respectively, scored. Scale bar: 10 μm.

POP-1 activates Wnt-responsive genes in the endoderm founder blastomere, E

Our microarray results, however, presented a conundrum. Although sdz-23 and sdz-26 are derepressed in the MS blastomere in pop-1 mutant embryos and therefore expressed in both the MS and E lineages, the microarray results indicated, surprisingly, that these two genes were downregulated in pop-1 mutant twelve-cell embryos compared to wild-type twelve-cell embryos (Robertson et al., 2004). This troubling inconsistency was resolved when

carefully staged wild-type and pop-1 mutant embryos, each carrying the GFP reporter transgene, were examined side by side under the fluorescent microscope. Although similar GFP expression levels from the reporter transgene were observed in both MS and E lineages in pop-1 mutant embryos, these expression levels were significantly reduced when compared to the E-restricted expression levels in wild-type embryos (Figs. 1e, j, p). We observed similar results with end-1 and end-3 reporter GFP (Figs. 1o, p and not shown). We draw two main conclusions from these results. First, POP-1 not only represses the expression of E-specific

genes in MS, but also plays a positive role in activating their expression in the E blastomere. Second, in the absence of POP-1, these normally E-specific genes exhibit low, presumably basal, level expression in both MS and E.

sdz-23 and *sdz-26*, like *end-1* and *end-3*, are not only expressed in an E-specific manner, but are also Wnt-responsive genes. Expression from promoter fusion reporters for all four of these genes is abolished or dramatically reduced when WRM-1/β-catenin is depleted by RNAi (Figs. 2a–d and data not shown). The repression of E-specific genes observed in *wrm-1(RNAi)* embryos is dependent upon POP-1 protein. Removal of POP-1 in *wrm-1(RNAi)* embryos restores the expression of these genes to the low basal level seen in *pop-1(zu189)* or *pop-1(RNAi)* embryos (Figs. 2e–h). This result suggests that, although basal level expression of these Wnt-responsive genes does not require POP-1 protein, their expression can be further repressed or activated by POP-1. Wnt signaling switches POP-1, in a β-catenin-dependent manner, from being a negative regulator to being a positive regulator of the expression of these Wnt-responsive genes.

Both activation of *end-1* in E and repression in MS require direct binding by POP-1 to the *end-1* promoter

To further test the mechanism by which POP-1 positively regulates the expression of Wnt-responsive genes, we examined whether regulation of expression requires direct POP-1 binding to their promoter. Two optimal TCF binding sites, G(A/T)(A/T)CAAAG, are found in the genomic promoter fragment used to generate the *end-1* GFP reporter—GTTCAAAG beginning at −164 relative to the AUG translation initiation and GAACAAAG at −953. We mutated each site individually to G(A/T)(A/T)C*GGGG*, a change shown to abolish binding by the HMG domain (Korswagen et al., 2000). The *end-1* reporter with the −164 TCF binding site mutated exhibited low level basal expression in both the MS and E lineages (Figs. 2i, j), similar to the expression observed for the wild-type *end-1* reporter in the *pop-1* mutant. This low level expression is still detected in *wrm-1(RNAi)* embryos (Figs. 2k, l), consistent with our conclusion above that both activation and repression of a Wnt-responsive gene are POP-1-dependent. This result demonstrates that activation of *end-1* in E and repression of *end-1* in MS both require direct binding by POP-1 to a target site in the *end-1* promoter. The genomic promoter fragments used to evaluate *sdz-23* or *sdz-26* do not contain optimal TCF binding sites, suggesting that these two genes may not be direct targets of POP-1.

Activation of the Wnt-responsive genes requires the N-terminal β-catenin-binding domain of POP-1

Both fly and vertebrate TCF/LEF proteins activate Wnt-responsive genes following binding of β-catenin to the N-terminal domain of the TCF protein (Behrens et al., 1996; Brunner et al., 1997; Molenaar et al., 1996). Deletion of this N-terminal domain has a dominant negative result, as the
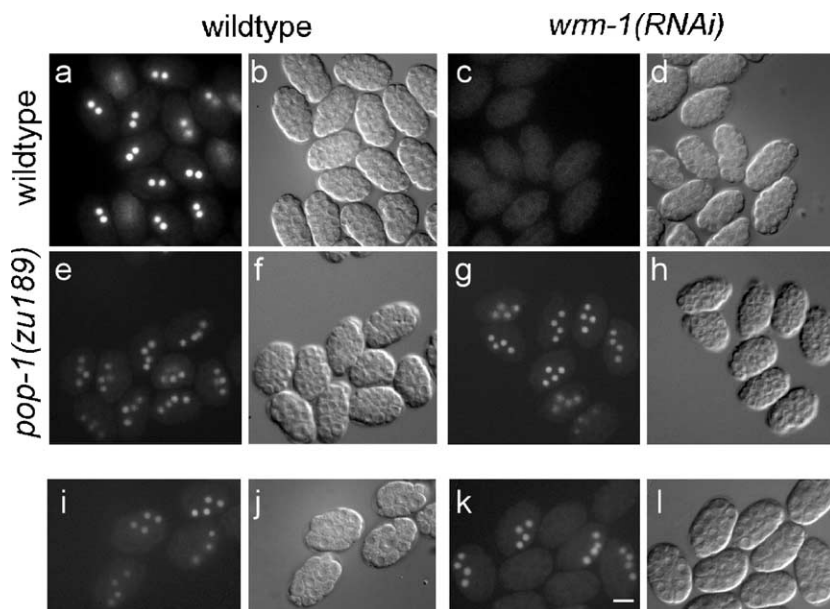

Fig. 2. Wnt signaling switches POP-1 from repressor to activator. (a–h) *sdz-23* reporter expression in *pop-1(+)* (wild-type; a–d) or *pop-1(zu189)* (e–h) backgrounds. Embryos shown in panels (c), (d), (g), and (h) were depleted of *wrm-1* by RNAi. The activation observed in wild-type embryos (a) and the repression observed in the *wrm-1(RNAi)* embryos (c) both require POP-1 (e.g., 'basal' level expression). (i–l) *pop-1(+)* embryos expressing an *end-1* reporter gene with the putative HMG-binding site at −164 mutated to GTTCGGGG in either non-RNAi (i, j) or *wrm-1(RNAi)* (k, l) backgrounds. 'Basal' level expression in both MS and E lineages is observed. This transgene is an extrachromosomal array and is not integrated. All embryos shown at 2E and 2MS stage. Scale bar: 20 μm.

TCF protein remains bound by corepressors (Cavallo et al., 1998; Roose et al., 1998). We and others have previously shown the N-terminal domain of POP-1 (amino acids 1 through 47) to be dispensable for both lowered POP-1 nuclear levels in E and rescue of the MS defect in *pop-1(zu189)* mutant embryos (Lo et al., 2004; Maduro et al., 2002). The rescue of the MS defect, however, only scores the ability of POP-1 to repress the endoderm fate (i.e. the ability to repress E-specific genes in MS) and does not address potential activation of E-specific genes by POP-1 in E.

To test whether the activation of Wnt-responsive genes in the E blastomere requires the N-terminal domain of POP-1, we used a *pop-1(zu189)* strain containing an integrated $P_{sdz-23}gfp::H2B$ reporter. Following the introduction of either full-length POP-1 or ΔN POP-1 (Δ1–47) into this

strain, we assayed the effect upon reporter gene expression (Figs. 3a, b, e, f). Consistent with previous findings that the N-terminal domain is dispensable for POP-1 rescuing activity (Lo et al., 2004; Maduro et al., 2002), both POP-1 constructs repress the expression of the GFP reporter in MS. On the contrary, while full-length POP-1 restores a high level expression of the GFP reporter in E, ΔN POP-1 does not. A similar effect was observed with POP-1 in which an N-terminal invariant aspartate was mutated to glutamate (D_9E, Figs. 3c, g). This aspartate has been shown to be required for β-catenin binding and the β-catenin-dependent transcription activation of vertebrate TCF proteins (Graham et al., 2000; Hsu et al., 1998; von Kries et al., 2000). This D_9E change corresponds to the *pop-1(q645)* mutation, which was isolated as a reduction-of-function mutation affecting somatic gonad
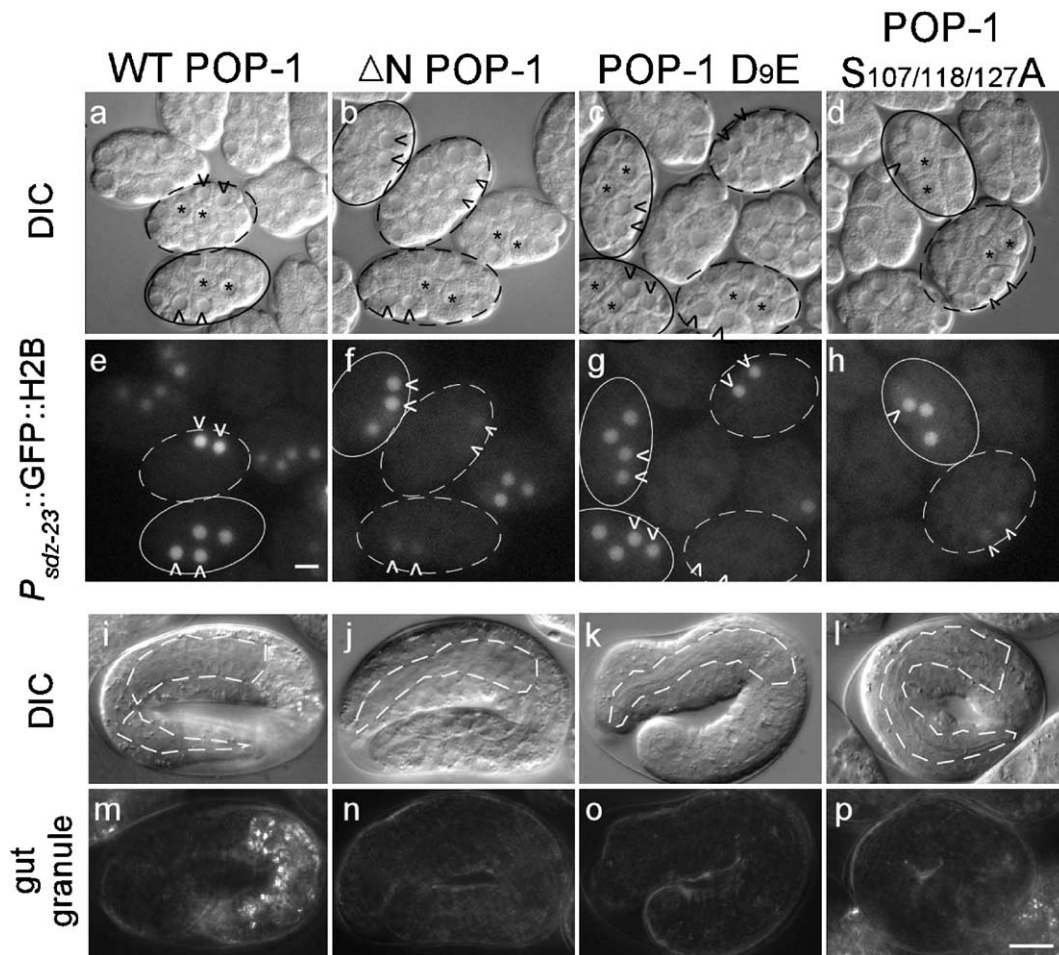

Fig. 3. Activation of E-specific genes requires both the N-terminal domain of POP-1 and a low nuclear POP-1 level. *pop-1(zu189)* with integrated $P_{sdz-23}gfp::H2B$ also expressing either CFP- or YFP-fused full-length wild-type POP-1 (a, e, i, m), N-terminal domain depleted POP-1 (b, f, j, n), POP-1 D_9E (c, g, k, o) or POP-1 $S_{107/118/127}A$ (d, h, l, p) (see Materials and methods). The transgenes expressing POP-1 variants are not integrated. (a–h) DIC images of embryos above corresponding GFP::H2B fluorescence micrographs. Embryos expressing the *pop-1* transgene are in dashed outline, while embryos lacking transgene expression are in solid outline. Open arrowheads: Ea and Ep. Asterisks: MSa and MSp. Whereas full-length POP-1 and all three variants are capable of repressing GFP expression in the MS lineage, only full-length wild-type POP-1 is capable of restoring high level GFP expression in the E lineage (e; observed in 44% of embryos expressing POP-1). (i–l) All four POP-1 versions rescue the MS defect (i.e. restore generation of posterior pharynx). Dashed outline: pharynx. (m–p) All embryos expressing full-length wild-type POP-1 develop endoderm (as evidenced by gut granule birefringence, m), whereas a proportion of embryos expressing the other three POP-1 mutant proteins are endoderm ablated (n, o, p). Similar results were obtained using a strain carrying integrated $P_{end-1}gfp::H2B$ (not shown). Scale bar: 10 μm.

morphogenesis, a larval Wnt-dependent process that requires WRM-1 (Siegfried and Kimble, 2002). Of embryos expressing ΔN POP-1 or POP-1 D$_9$E, 71 and 50%, respectively, have GFP expression levels repressed to below the basal level seen in the *pop-1(zu189)* mutant (Figs. 3f and g). A dominant negative effect in endoderm formation was also observed in those embryos with reduced GFP expression levels, with some embryos exhibiting complete endoderm ablation (19% and 15%, respectively; Figs. 3i–k, m–o). This dominant negative effect suggests that, while the N-terminal β-catenin binding domain of POP-1 is not required for repression, it is required for the Wnt-dependent activation of E-specific genes. Given the requirements for the N-terminal domain of POP-1, particularly aspartate at position nine, it is likely that this activation requires a β-catenin as coactivator.

Activation of Wnt-responsive genes in E requires lowered nuclear POP-1 levels

These results and our reinterpretation of Wnt signal-induced endoderm formation raise a number of new questions. Why are POP-1 levels in the E nucleus lowered in response to Wnt signal, if we now propose that POP-1 functions as a transcriptional activator in the Wnt-responsive cell? To address this question, we used a POP-1 mutant that exhibits high nuclear levels in Wnt-responsive cells. We have shown previously that this POP-1 variant (POP-1 S$_{107/118/127}$A) is present at a high level in the E nucleus due to a defect in Wnt-induced nuclear export (Lo et al., 2004). Nevertheless, this export-defective POP-1 protein was able to fully rescue the MS defect in *pop-1(zu189)* mutants (Lo et al., 2004). We find that this export-defective POP-1 protein represses Wnt-responsive genes in the MS blastomere but fails to activate them in E (Figs. 3d, h). In fact, expression of this export-defective POP-1 protein often represses the Wnt-responsive gene expression levels in E to below the basal level and has a dominant negative effect on endoderm formation (60% of embryos, Figs. 3l, p). This result demonstrates not only that Wnt signaling lowers the nuclear POP-1 level in the Wnt-responsive cell, but also that a lowered POP-1 level is required for activation of Wnt-responsive genes in E. We believe that POP-1 levels are reduced to effect a change in the POP-1:coactivator ratio that favors gene activation over repression in a cellular environment where coactivator is limiting and unchanged following Wnt signal (see further below). Our results provide an alternative to the commonly accepted model for endoderm induction in *C. elegans*. Rather than lowered nuclear POP-1 levels in E resulting in simple derepression of endoderm-specifying genes, we now show that POP-1, when reduced in level in the E nucleus, acts as an activator of these genes.

Endoderm is still induced in *pop-1(zu189)* or *pop-1(RNAi)* embryos (Lin et al., 1995), demonstrating that, in the absence of POP-1, simple derepression and basal level expression of endoderm-specific genes in the E

nucleus is sufficient to induce endoderm formation. Indeed, the observation that basal level expression of Wnt target genes in *pop-1* mutant embryos was sufficient to induce endoderm is the likely reason why the role of POP-1 in the activation of Wnt-responsive genes has gone undetected until now. So why go to the bother of converting POP-1 into an activator of these genes at all? We suggest that endoderm formation is only a partial readout of the effect of Wnt signaling on the E blastomere and that Wnt signaling may also change properties of the E blastomere independent of E-derived endoderm formation, such as how E and/or E descendants interact with neighboring blastomeres. This proposal requires that some of the genes exhibiting E lineage-specific expression do not function directly in endoderm specification or elaboration of the intestine. In fact, no defect in endoderm formation was detected when *sdz-23*, *sdz-26* or both were depleted by RNAi (data not shown). The fact that *sdz-23* encodes a variant Notch ligand (Robertson et al., 2004) is intriguing in this regard, and a possible function in cell–cell interactions is currently under investigation.

Although Wnt-induced lowering of nuclear TCF protein has only been demonstrated unambiguously in *C. elegans*, TCF protein levels clearly can affect Wnt signaling in the canonical pathway. The strength of Wnt signaling in flies has been shown to be determined, at least in part, by relative levels of both Pangolin/dTCF and Armadillo/β-catenin (Cavallo et al., 1998; Schweizer et al., 2003). That is, a lower level of dTCF or a higher level of ARM seems to contribute to strong Wnt signaling. In *Drosophila*, *wingless* and *armadillo* mutant phenotypes can be partially suppressed by a reduction in dTCF activity, whereas the phenotype of a weak *wingless* allele is enhanced by overexpression of wild-type dTCF (Cavallo et al., 1998). This is reminiscent of our observation here and suggests that determination of Wnt signal strength, in part by the relative levels of TCF/LEF transcription factor and relevant cofactors, is a more general aspect of canonical Wnt signaling.

Although WRM-1 is the only β-catenin shown to function in endoderm induction (Rocheleau et al., 1997), there is no evidence suggesting that it does so by functioning as a coactivator for POP-1 in the expression of Wnt-responsive E-specific genes. WRM-1 interacts with POP-1 only very weakly (Natarajan et al., 2001; Rocheleau et al., 1999), and this weak interaction is not dependent on the POP-1 β-catenin-binding domain (Natarajan et al., 2001). The essential role of WRM-1 in lowering POP-1 nuclear levels alone can account for the *wrm-1(RNAi)* embryo phenotype (Rocheleau et al., 1999). BAR-1, on the other hand, binds to POP-1 but plays no demonstrable role in endoderm induction (Eisenmann et al., 1998; Korswagen et al., 2000; Natarajan et al., 2001). These are all highly variant β-catenins—whereas *Drosophila* Armadillo is 67% identical to human β-catenin, the three worm proteins range from 17 to 27% identity and share even less identity when

compared among themselves. There are other Armadillo repeat-containing proteins encoded within the *C. elegans* genome, and whether one or more of them functions as the coactivating β-catenin for POP-1 in this process remains to be determined.

Model

Therefore, we propose a modified version of the canonical Wnt signaling pathway which explains the requirement for lowered POP-1 levels in the E nucleus in response to Wnt signal. In the absence of Wnt signaling, TCF protein is present in the nucleus in excess of limiting amounts of coactivator, its preferred binding partner (Herman, 2001; Siegfried et al., 2004). The excess nuclear TCF protein

binds to corepressors and represses transcription of target genes. Wnt signaling switches TCF protein to a transcriptional activator of target genes by increasing the amount of coactivator in the nucleus relative to TCF protein, thereby allowing most or all of the nuclear TCF protein to be complexed with coactivator. Wnt signaling increases the coactivator to TCF ratio either by increasing the level of nuclear coactivator (as in the canonical Wnt model, Fig. 4B), by decreasing the amount of nuclear TCF protein (as observed in the *C. elegans* endoderm founder, E; Fig. 4C) or both.

The data presented here clarify the role of the *C. elegans* TCF/LEF protein POP-1 in Wnt-induced endoderm formation in the early embryo. Rather than invoking, as some have termed, a non-canonical Wnt pathway based upon
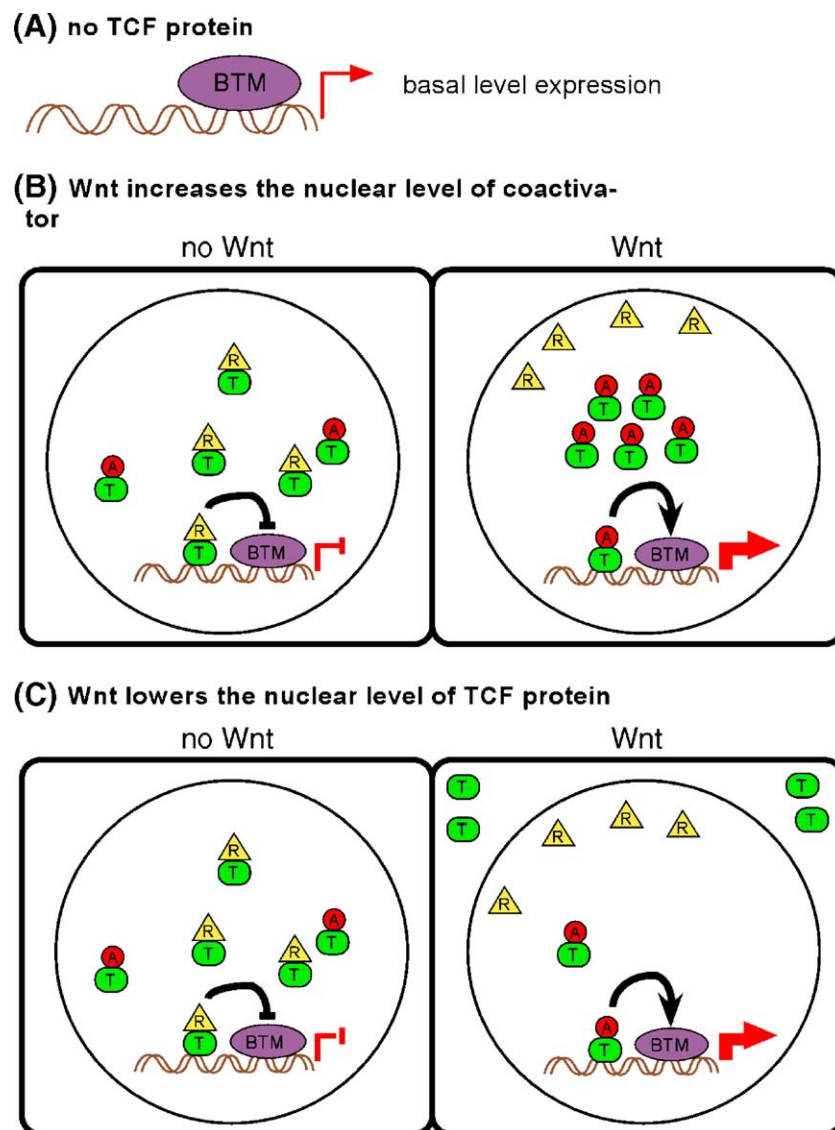

Fig. 4. Model for how Wnt signaling activates target gene expression. (A) In the absence of TCF protein, basal transcription machinery (BTM, purple oval) and other ubiquitous transcription factors engage the promoter for Wnt-responsive genes, resulting in low-level basal expression. (B, C) In the absence of Wnt signaling (cells on the left), TCF protein (green oval) complexes with corepressor(s) (yellow triangle) to repress the expression of Wnt target genes to below the basal level. Wnt signaling (cells on the right) activates the expression of target genes by increasing the nuclear level of coactivator (red circle) relative to TCF protein. This can be achieved by an increase in the nuclear level of coactivator (B), decreasing the nuclear level of TCF protein (C) or both.

TCF/LEF derepression (Herman, 2002; Herman and Wu, 2004; Korswagen, 2002), we now show that endoderm specification in *C. elegans* results from a variation of the canonical pathway. In both the canonical pathway and endoderm specification in the worm, the end result is the same: activation of Wnt-responsive target genes via preferred interaction between TCF/LEF transcription factors and coactivator(s) versus corepressor(s). The requirement for lowered nuclear TCF/LEF levels in the Wnt responsive E blastomere, along with supporting evidence from *Drosophila*, highlights the importance of levels of TCF/LEF relative to corepressors and coactivators in determining Wnt signal strength.

Acknowledgments

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