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Dynamics of a Developmental Switch: Recursive Intracellular and Intranuclear Redistribution of *Caenorhabditis elegans* POP-1 Parallels Wnt-Inhibited Transcriptional Repression

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POP-1, a Tcf/Lef factor, functions throughout *Caenorhabditis elegans* development as a Wnt-dependent reiterative switch to generate nonequivalent sister cells that are born by anterior–posterior cell divisions. We have observed the interaction between POP-1 and a target gene that it represses as it responds to Wnt signaling. Dynamic observations in living embryos reveal that POP-1 undergoes Wnt-dependent nucleocytoplasmic redistribution immediately following cytokinesis, explaining the differential nuclear POP-1 levels in nonequivalent sister cells. In unsignaled (anterior) but not Wnt-signaled (posterior) sister cells, POP-1 progressively coalesces into subnuclear domains during interphase, coincident with its action as a repressor. While the asymmetric distribution of POP-1 in nonequivalent sisters apparently requires a 124-amino-acid internal domain, neither the HMG box nor β-catenin interaction domains are required. We find that a transcriptional activator, MED-1, associates *in vivo* with the *end-1* and *end-3* target genes in the mesoderm (anterior sister) and in the endoderm (posterior sister) following the asymmetric cell division that subdivides the mesendoderm. However, in the anterior sister, binding of POP-1 to the *end-1* and *end-3* genes blocks their expression. *In vivo*, binding of POP-1 to the *end-1* and *end-3* targets (in the posterior sister) is blocked by Wnt/MAPK signaling. Thus, a Tcf/Lef factor represses transactivation of genes in an unsignaled daughter cell by abrogating the function of a bound activator.

Key Words: **Wnt; MAP kinase; POP-1; Lef-1; asymmetric cell division; transcription.**

INTRODUCTION

Members of the TCF/LEF family of HMG box proteins mediate the transcriptional regulatory output of Wnt/Wingless signaling in diverse systems (reviewed in Cadigan and Nusse, 1997; Roose and Clevers, 1999). In the canonical Wnt pathway, signaling inhibits degradation of cytosolic β-catenin, which then enters the nucleus and binds to members of the TCF/LEF family. The β-catenin–TCF/LEF combination comprises a bipartite transcription factor that activates target genes. In the absence of signaling, TCF/LEF proteins can function as repressors through their association with Groucho-like corepressors (Cavallo *et al.*, 1998; Brantjes *et al.*, 2001).

The TCF/LEF factor, POP-1, plays a prominent role throughout *Caenorhabditis elegans* development as a regulatory switch responsible for reiteratively establishing differences between daughter cells that arise from developmentally asymmetric cell divisions (Lin *et al.*, 1998). POP-1 makes the first of its numerous appearances as such a regulatory switch during establishment of the endoderm in the early embryo. At the four-cell stage, the ventralmost blastomere, EMS, becomes polarized through contact with its neighbor, P_2, such that the side of EMS in contact with P_2 becomes the E cell when EMS divides (Goldstein, 1993, 1995). This intercellular signal is mediated by an overlapping Wnt/MAPK signaling system (Thorpe *et al.*, 1997; Rocheleau *et al.*, 1997, 1999; Shin *et al.*, 1999; Schlesinger *et al.*, 1999). In the absence of this signal, EMS divides to produce two MS-like daughters and no endoderm is made

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(Goldstein, 1993; Thorpe *et al.,* 1997; Rocheleau *et al.,* 1997). Removal of maternal POP-1 leads to the opposite phenotype: EMS instead divides to produce two E-like daughters (Lin *et al.,* 1995). These findings indicate that POP-1 represses endoderm fate in MS, while in E, Wnt/MAPK signaling blocks its repressive function (Thorpe *et al.,* 1997; Rocheleau *et al.,* 1997; Fig. 1).

Immunostaining of POP-1 revealed that it is present in many cells throughout development (Lin *et al.,* 1995, 1998). In sister cells arising from cell division along the anterior–posterior (A/P) axis, including MS and E, POP-1 immunostaining is detectable at higher levels in anterior compared to posterior nuclei (Lin *et al.,* 1998), suggesting either differential stability or localization of the protein, or, more trivially, masking of immunoreactive protein in posterior nuclei. Depletion of upstream Wnt/MAPK signaling components eliminates this "POP-1 asymmetry," resulting in symmetrical staining of POP-1 in MS and E, and other A/P sister pairs (Lin *et al.,* 1998; Rocheleau *et al.,* 1999; Shin *et al.,* 1999). Expression of high levels of POP-1 is correlated with anterior fates in many lineages, in both the early embryo and in postembryonic cell divisions, and in many of these cases has been shown to be required for A/P differences (Lin *et al.,* 1995, 1998; Jiang and Sternberg, 1999; Korswagen *et al.,* 2000; Herman, 2001). The mechanism by which nuclear POP-1 levels become different in anterior (unsignaled) and posterior (Wnt/MAPK-signaled) cells has not been determined.

To reveal the mechanisms by which POP-1 reiteratively establishes differences between nonequivalent sisters, it is necessary to observe its *in vivo* behavior and interactions with its regulatory targets. The best candidates for direct POP-1 targets are the *end-1* and *-3* genes, which encode redundant GATA-type transcription factors that zygotically specify the identity of the E cell (Zhu *et al.,* 1997; M.M. and J.R., unpublished observations). Activation of *end-1, -3* requires the redundant, zygotically expressed GATA factors MED-1, -2 (Maduro *et al.,* 2001), while repression of endoderm fate in the MS blastomere requires maternally provided POP-1 (Lin *et al.,* 1995). Vertebrate TCFs can mediate repression by recruitment of histone deacetylases to promoters via Groucho family corepressors (Roose *et al.,* 1998; Chen *et al.,* 1998; Brantjes *et al.,* 2001). POP-1 represses endoderm fate in a complex consisting of a Groucho-like protein (UNC-37) and a histone deacetylase (HDA-1), showing that POP-1 represses by an evolutionarily conserved mechanism (Calvo *et al.,* 2001). In a *pop-1(−)* mutant, MED-1, -2 are required for the endoderm that arises from both MS and E (Maduro *et al.,* 2001). This suggests that activation by MED-1, -2, combined with the asymmetry provided by POP-1, dictates either the MS fate (MED-1, -2 + high POP-1) or E fate (MED-1, -2 + low, or Wnt-modified, POP-1) (Maduro *et al.,* 2001).

Here, we reveal the dynamic behavior of POP-1 as a developmental switch in Wnt/MAPK-signaled and -unsignaled sister cells within living embryos. We find that Wnt/MAPK-dependent "POP-1 asymmetry" is the result of the nucleocytoplasmic redistribution of POP-1 in the signaled sister immediately upon its birth, rather than differences in the total levels of POP-1. This asymmetric intracellular distribution of POP-1 apparently requires an internal 124-amino-acid region, but not the β-catenin or HMG box (DNA binding) domains. We find that in the absence of Wnt/MAPK signal, POP-1 undergoes a progressive relocalization into subnuclear domains during each interphase following cell division. These findings suggest qualitative differences between signaled and unsignaled POP-1. Indeed, by observing direct protein-DNA interactions in individual nuclei of living embryos, we find that these differences are correlated with the ability of POP-1 to bind directly to two of its targets, the *end-1* and *end-3* genes. While POP-1 binds *end-1, -3* in unsignaled (anterior) cells, it is not detectably bound in Wnt/MAPK-signaled cells. We find that MED-1 associates directly with *end-1* and *-3 in vivo* in all early EMS descendants, showing that POP-1 represses target gene expression by inhibiting the activity of a bound activator rather than precluding its binding. These findings suggest that a TCF/LEF factor, POP-1, reiteratively establishes differences in sister cells by Wnt/MAPK-dependent alterations in its intracellular and intranuclear distribution and DNA-binding properties.

MATERIALS AND METHODS

Plasmids and Cloning

DNA manipulation was performed according to standard protocols. All pPD vectors were obtained from Andrew Fire. The mgPOP-1 and *med-1*::cmyc::POP-1 and similar constructs were made from *med-1* reporters (Maduro *et al.,* 2001) by replacing *med-1* coding sequences with PCR-amplified segments from a *pop-1* cDNA. Reporters containing GFP fused to the carboxyl terminus of POP-1 do not recapitulate POP-1 asymmetry (data not shown). Mutant versions of mgPOP-1 were built either by removing intervening restriction fragments or by using PCR-based strategies. Double-stranded RNAs were synthesized by using the T7 MEGAscript kit (Ambion) from PCR products amplified by primers tagged with T7 promoter sequences. Oligonucleotide sequences and cloning details are available on request.

C. elegans Strains and Genetics

Growth of *C. elegans* strains was performed according to standard procedures. For transgenics or RNAi, gonadal injection of DNA (~100 ng/μL each plasmid) and RNA (~5 μg/μL) were performed as described (Mello *et al.,* 1991). In some cases, injected hermaphrodites were mated with wild-type or *him-8(e1489)* males to increase brood size. Constructs were coinjected with either the dominant *rol-6* marker pRF4 or the *unc-119* rescuing plasmid pDP#MM016B into wild-type (N2) hermaphrodites or *unc-119(ed4)* mutants, respectively. Strains containing two transgene arrays were constructed by combining *unc-119*-rescued and *rol-6(d)*-marked lines as described (Maduro *et al.,* 2001). The mgPOP-1 integrant *wls117* was isolated after γ-irradiation (3000 rad) of an unintegrated [pMM414 + pRF4] array strain, and screening of F_2 progeny for 100% Rol. Expression of *wls117, wls117/+,* and unin-

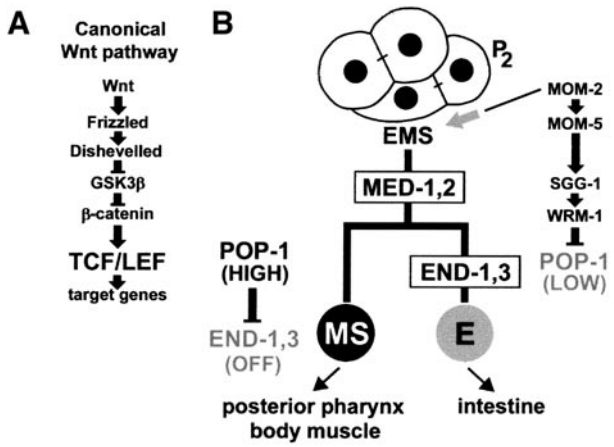

FIG. 1. Model for specification of E and MS fates. (A) Abbreviated canonical Wnt pathway (after Herman, 2001). (B) The developmental switch that directs mesendoderm development. Zygotic expression of *med-1, -2* is activated at the four-cell stage in EMS, the parent of MS and E. In MS, POP-1 blocks expression of the E-promoting genes *end-1* and *end-3*. In E, modification of POP-1 by Wnt/MAPK signaling, via the WRM-1/LIT-1 kinase, blocks the repressive activity of POP-1, allowing MED-1, -2 to activate *end-1, -3* expression. The MS nucleus shows stronger immunostaining than the E nucleus (shown as differential shading), similar to POP-1 staining in other anterior–posterior sisters. In the diagrammed four-cell-stage embryo, anterior is to the left, and dorsal is up.

tegrated reporters marked with either *rol-6(d)* or *unc-119(+)* rescue was qualitatively similar.

To introduce *wls117* into a *lit-1* background, males from strain GE2244 [*unc-32(e189) lit-1(t1512)/qC1 dpy-19(e1259) glp-1(q339) III; him-3(e1147) IV*] were crossed into *unc-119(ed3); wls117* hermaphrodites. F_2 Rol Unc-32 homozygotes were picked, and those segregating 100% dead eggs at 25°C were kept. A similar strategy was used for *lit-1(t1534)*.

Detection of Extrachromosomal Arrays

Target *end-1* or *end-3* arrays were generated by coinjection of an *end-1*::lacZ or *end-3* promoter clone with a *lacO* multimeric plasmid (pSV2-dhfr-8.32; a gift from Andrew Belmont), heat shock myc-*lacI* plasmids, and *unc-119(+)* plasmid pDP#MM016B (Maduro and Pilgrim, 1995). Two hs-NLS-cmyc-lacI constructs were built in steps, using the heat-shock promoters *hsp-16-2* and *hsp-16-41* (from pDP49.78 and pPD49.83, respectively), the SV40 nuclear localization signal (NLS) from pPD95.67, the LacI ORF (lacking the last six amino acids) amplified from a wild-type *Escherichia coli* strain, and the 30 carboxyl-terminal amino acids of c-myc amplified from plasmid pUHE2 (a gift from David Low). To induce *lacI* expression, transgenic animals were given a 30-min heat shock at 33°C, and fixed after a further 30 min at 22°C.

Transgene POP-1 Rescue

Rescue of *pop-1(zu189)* was measured as follows. Males from a *him-8(e1489); unc-119(ed3); wEx*[mgPOP-1 + *unc-119(+)*] strain were mated to Dpy progeny from *pop-1(zu189) dpy-5(e61)/hT1* hermaphrodites. Only progeny embryos that had undergone extensive morphogenesis, in which the pharynx is easily visualized, were scored. Mating of nontransgenic males with *pop-1(zu189)* m+z− homozygotes does not produce rescue of m−z+ progeny (Lin *et al.*, 1995). To confirm rescue by full-length mgPOP-1 in *pop-1* m−z−, males from the strain *pop-1(zu189) dpy-5(e61)/hT1 I; him-5(e1489)/hT1 V* were mated with *wls117* hermaphrodites, Rol F_1s were selfed, and Dpy F_2s were singled. Plates on which the vast majority of F_3 progeny embryos failed to hatch [indicating the presence of *pop-1(zu189)*] were scored for pharynx by Nomarski optics: 9% (29/332) contained a complete pharynx, and 17% (5/29) of these hatched to produce arrested L1 larvae. This approach yields an underestimate for rescue, because not all embryos scored carry the *wls117* marker, and because many *pop-1(zu189)* embryos do not undergo morphogenesis (Lin *et al.*, 1995). Attempts to deplete endogenous POP-1 by RNAi in the mgPOP-1 strain by targeting the *pop-1* 3′UTR were not successful, perhaps because the *pop-1* 3′UTR adopts a secondary structure *in vivo* (Morse and Bass, 1999).

In Situ Hybridization

Detection of the *wls117* and *end-3*::GFP transgene messages was performed by using the *in situ* hybridization protocol of Seydoux and Fire (1995) with an antisense GFP probe amplified from pPD79.44.

Immunohistochemistry and Fluorescence Microscopy

Detection of POP-1 was performed by using mAbRL2 as described (Lin *et al.*, 1998). For detection of *cmyc* or GFP, embryos were prepared as described (Zhu *et al.*, 1997) and stained using an anti-myc monoclonal antibody (9E10; Boehringer Mannheim) or a chicken anti-GFP (AB16901; Chemicon) with Jackson Immunolabs or Sigma secondary antibodies, all at a dilution of 1:200. Anti-GFP or anti-POP-1 staining of GFP::POP-1 gave A/P results similar to GFP fluorescence in live embryos, except that the puncta seen in living embryos were difficult to see after fixation. Anti-POP-1 staining of the mgPOP-1 strain showed ~10× brighter signal from anterior nuclei in the EMS lineage compared with anterior nuclei in other lineages. Polyclonal antisera raised against Histone H4 peptides whose sequences are conserved in *C. elegans* was obtained from Serotec (#PAN015; includes antisera to nonacetylated Histone H4, fully acetylated, and acetylated at positions 5, 8, 12 or 16). Images were acquired by using a Bio-Rad 1024 Confocal Microscope or a DVC digital camera on a Zeiss Axioskop 2. Fluorescence images from multiple focal planes were combined and enhanced for contrast by using Confocal Assistant 4.02 (T. C. Brelje) and Adobe Photoshop 6. Pseudocolored images were made with the "thermo" lookup table in Confocal Assistant.

RESULTS

Zygotic Expression of POP-1 in EMS Is Sufficient to Restore MS Development in pop-1 Maternal Mutants

We established a system for analyzing the *in vivo* dynamics of POP-1 during developmentally asymmetric cell divisions by examining the behavior of a GFP-tagged POP-1 chimera in living embryos. As expression of maternal genes, such as *pop-1*, is difficult to achieve from transgenes,

we expressed the chimera from the promoter of *med-1*, one of the earliest expressed zygotic genes in *C. elegans*. *med-1* is first expressed in EMS (Maduro *et al.*, 2001); thus, the GFP::POP-1 might be expected to reveal the response of POP-1 to Wnt/MAPK signaling. We will refer to this construct as mgPOP-1 (for *med-1*-driven GFP::POP-1; Fig. 2A).

We found that, although POP-1 is maternally required for MS specification (Lin *et al.*, 1995), mgPOP-1 expressed zygotically in EMS was nonetheless capable of providing this POP-1 function. In embryos lacking maternal *pop-1* activity, the MS-derived portion (posterior half) of the pharynx is absent and such embryos arrest with ectopic endoderm as the result of a transformation of MS into an E-like cell (Fig. 2B; Lin *et al.*, 1995). Among *pop-1(−)* embryos expressing mgPOP-1, in which the anterior half of the pharynx could clearly be recognized, ~60% (n = 58; adjusted for transmission frequency of the transgenic array) also contained a posterior pharynx (Fig. 2C). Moreover, the intestine appeared normal in these rescued animals, demonstrating that the Wnt/MAPK machinery is capable of blocking the repressive function of transgenic mgPOP-1 in E. The failure of the transgene to rescue the *pop-1(−)* mutants to viability is expected since mgPOP-1(+) is supplied only in the EMS lineage, while POP-1 is also required in many other lineages (Lin *et al.*, 1998). We conclude that zygotically expressed POP-1 can recapitulate an asymmetric pattern sufficient to restore MS fate and that mgPOP-1 is a reliable *in vivo* marker for functional POP-1.

POP-1 Asymmetry between A/P Sisters Arises from Differential Nucleocytoplasmic Distribution

The finding that mgPOP-1 provides *pop-1(+)* rescuing activity allowed us to address several questions regarding reiterated POP-1 nuclear asymmetry: first, whether this asymmetry reflects genuine changes in the amount of protein (Lin *et al.*, 1995, 1998); second, whether this asymmetry results from differential stability, synthesis, or nuclear transport (Rocheleau *et al.*, 1999; Thorpe *et al.*, 2000), and third, how POP-1 levels change dynamically in response to a Wnt/MAPK signal following an asymmetric cell division.

We found that mgPOP-1 recapitulates the asymmetric nuclear levels in sister cells of A/P cell divisions seen by immunodetection of the protein (Lin *et al.*, 1995, 1998). mgPOP-1 is present at higher levels in anterior daughters of A/P divisions at several sequential stages in the EMS lineage even when the transgene mRNA is no longer detectable (see below; Figs. 3A–3C; and Table 1). Quantification of the mid-interphase pixel intensities between A/P nuclei in multiple lineages demonstrated that the signal difference is approximately twofold (see Materials and Methods). As seen with immunostaining of endogenous POP-1, β-catenin/WRM-1 and other Wnt/MAPK components are required for this asymmetry in nuclear mgPOP-1 levels (Fig. 3D and Table 1; Thorpe *et al.*, 1997; Rocheleau *et al.*, 1997; Lin *et al.*, 1998). Interestingly, we observed symmetric expression of mgPOP-1 in a *lit-1(t1534)* strain, which nonetheless generally produces endoderm (Rocheleau *et al.*, 1999). This result was confirmed with anti-POP-1 immunostaining of *t1534* embryos (R.L., unpublished observations), suggesting that POP-1 asymmetry can be uncoupled from endoderm specification (see Discussion). Depletion of the CBP/p300 homolog CBP-1, or the histone deacetylase HDA-1 two maternal proteins that influence activation of *end-1, -3*, had no effect on mgPOP-1 asymmetry (Calvo *et al.*, 2001). These observations reveal that the reiterative POP-1 asymmetry observed between A/P sister cells reflects *bona fide* differences in nuclear protein levels, rather than differences in accessibility of an immunoepitope.

Given the robust signal detected from mgPOP-1 (expressed at least 10-fold greater than endogenous POP-1, as assessed by immunostaining of mgPOP-1 with anti-POP-1 sera; see Materials and Methods), we were able to ask whether the reduced nuclear POP-1 levels in posterior daughter cells might be explained by differences in protein stability or nuclear transport. In all embryos, we observe a low level of cytoplasmic mgPOP-1. Close examination of signal intensities from confocal microscopic images revealed a slight increase in the level of cytoplasmic signal in E, MSp, and Ep than in their anterior sisters (e.g., apparent in the E cell shown in Fig. 3B). When the signal intensities are adjusted for differences in volume between the nucleus and cytoplasm, we find that there is sufficient mgPOP-1 present in the posterior cytoplasm to account for the A/P nuclear differences between sister cells (Fig. 4): i.e., both anterior and posterior sister cells contain similar total amounts of mgPOP-1. Thus, the Wnt/MAPK pathway induces a change in the nucleus to cytoplasm distribution of POP-1, but apparently without significant degradation in either intracellular compartment.

mgPOP-1 made it possible to evaluate the dynamics of POP-1 asymmetry and thereby assess when the Wnt/MAPK signal effects this change in nuclear POP-1 levels. We followed changes in mgPOP-1 expression and localization in developing embryos beginning at the 16-cell stage, when the MS and E daughters are well into interphase. At this stage, *med-1* transcripts are undetectable by the sensitive method of RT-PCR, and mg*pop-1* transgene transcripts are no longer detectable by *in situ* hybridization, indicating that any changes in mgPOP-1 signal are independent of new synthesis of the protein (Maduro *et al.*, 2001; and data not shown). A representative series of time-lapse images is shown in Figs. 3I–3P. The onset of mitosis was apparent from the loss of a distinct nuclear envelope as seen by Nomarski microscopy, and by comparison with similar time-lapse recordings of a *pie-1*::GFP::HistoneH2B strain, which allows visualization of chromatin compaction (Praitis *et al.*, 2000). Throughout the cell cycle, low but significant levels of signal are visible in the cytoplasm. Striking nuclear A/P asymmetry is apparent almost immediately after cytokinesis, i.e., within seconds after the start of interphase (e.g., MS^4 in Fig. 3L). Unexpectedly, the

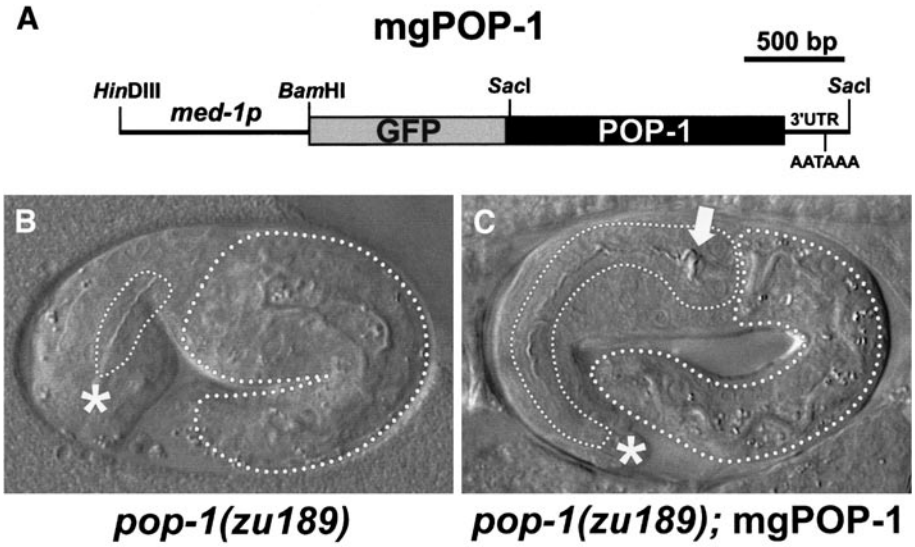

FIG. 2. Rescue of *pop-1(zu189)* by mgPOP-1. (A) Diagram of the mgPOP-1 transgene pMM414. (B) Arrested *pop-1(zu189)* mutant embryo showing abnormally small pharynx (thin dotted line) and excess intestine (thick dotted line). The anterior end of the pharynx, to which the buccal cavity would be attached in wild type, is indicated with an asterisk (*). (C) *pop-1(zu189)* mutant embryo carrying the mg*pop-1* transgene (integrant *wIs117*). This animal has elongated to approximately three times the length of the eggshell and contains a complete pharynx and normal intestine. The pharynx grinder, which is characteristic of posterior (MS-derived) pharynx, is indicated with an arrow. Elongation, as in this embryo, was observed in approximately 20% of rescued embryos; a small portion of these hatch to produce developmentally arrested L1 larvae. Anterior is to the left. A *C. elegans* embryo is approximately 50 μm in length.

mgPOP-1 levels in anterior sisters decrease at the end of interphase, such that A/P asymmetry is nearly undetectable immediately prior to mitosis (compare Ea and Ep in Fig. 3N). This observation underscores the benefits of the mgPOP-1 marker for assessing the dynamic behavior of the protein: the report that ~30% of embryos show equivalent levels of immunoreactive POP-1 in the E and MS nuclei (Lin *et al.*, 1995) is now likely explained by our finding that the amount of nuclear POP-1 in the anterior nucleus diminishes late in the cell cycle. In mitotic cells, POP-1 protein is not detectable by immunostaining (Lin *et al.*, 1995); however, we found that while nuclear mgPOP-1 disappears during mitosis, the substantial cytoplasmic fluorescence increases significantly (e.g., MS daughters in Fig. 3K). A/P differences in the cytoplasmic signal are not apparent during mitosis, further strengthening the notion that the total (nucleus + cytoplasm) amount of POP-1 is similar between sister cells. Cytoplasmic signal returns to premitotic levels at telophase, when the nuclear signal reappears (MS^4 in Fig. 3L). We conclude that POP-1 asymmetry is established very rapidly and is the result of nucleocytoplasmic redistribution of POP-1 protein.

The β-Catenin Binding Domain and HMG Box of POP-1 Are Dispensable for Asymmetry

We identified the broad structural requirements that allow POP-1 to respond to Wnt/MAPK signaling and shift its nucleocytoplasmic localization. POP-1 contains two features conserved with vertebrate TCF/LEF proteins: a centrally located DNA-binding HMG box, which shows the highest conservation (54% identity), and an N-terminal β-catenin interaction domain (Fig. 5A; Clevers and van de Wetering, 1997; Korswagen *et al.*, 2000). Of the three β-catenins in *C. elegans*, WRM-1, BAR-1, and HMP-2, only BAR-1 shows an interaction with POP-1 (Korswagen *et al.*, 2000). In analogy to the vertebrate LEF/TCF proteins, the first 44 amino acids of POP-1 are required for this interaction with BAR-1, suggesting that POP-1 is capable of a canonical β-catenin interaction (Korswagen *et al.*, 2000). We found that a form of mgPOP-1 lacking this amino-terminal domain exhibits asymmetric nuclear POP-1 that is indistinguishable from wild-type mgPOP-1 (Fig. 5B; and data not shown). This transgene is able to restore MS fate in a *pop-1* mutant, demonstrating that the β-catenin interaction domain is dispensable for the endoderm-repressing function of POP-1. This is consistent with our observation that *bar-1(RNAi)* embryos do not display defects in mgPOP-1 asymmetry and with the report that a putative *bar-1* null mutant does not display defects in MS specification (Table 1; Eisenmann *et al.*, 1998). As larger segments of POP-1 are deleted, the amount of nuclear mgPOP-1 signal becomes reduced in favor of increased cytoplasmic localization, making such fusions less informative (Fig. 5B). However, while a form of the protein lacking the HMG box is no longer able to rescue MS fate, both this truncation and one

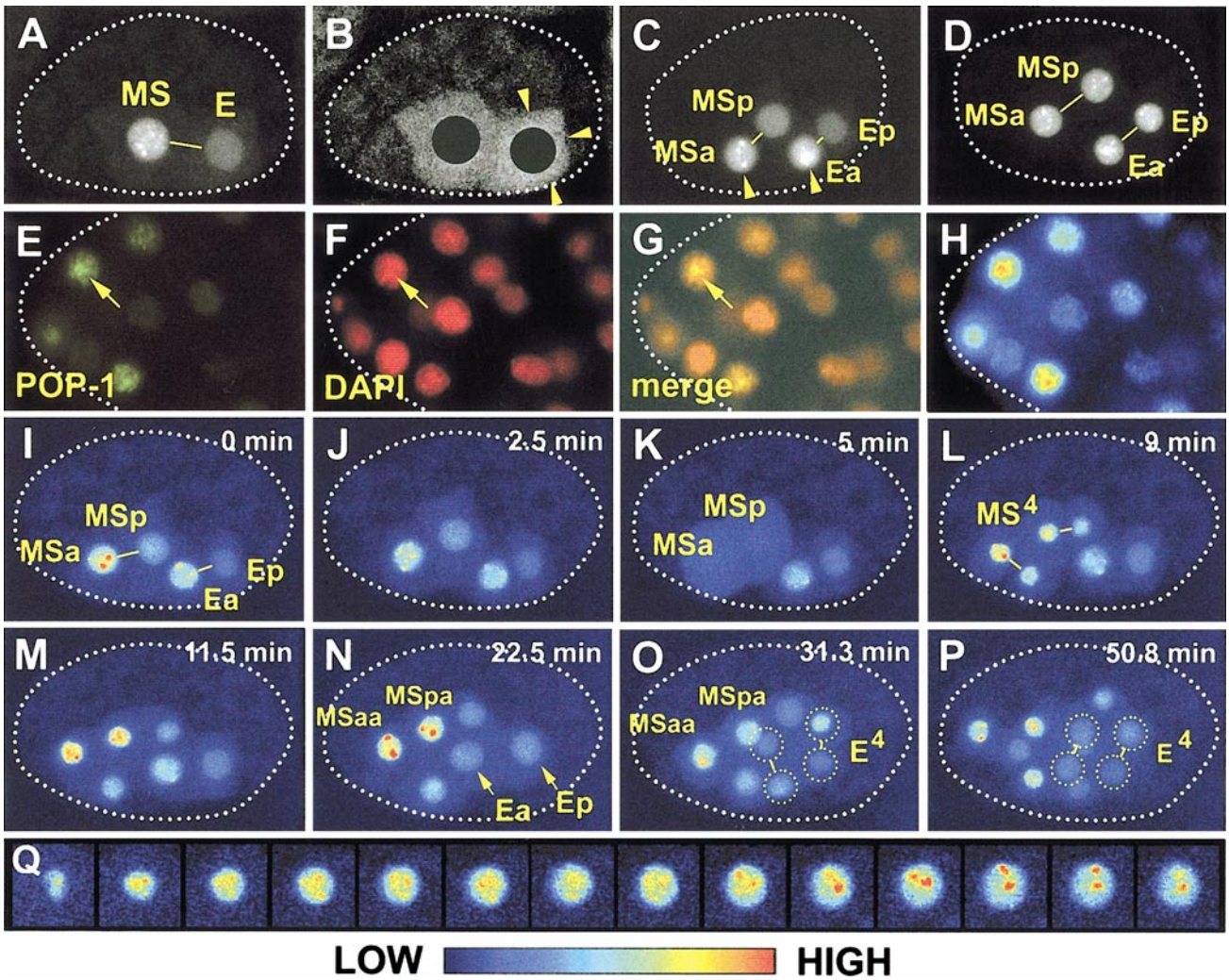

FIG. 3. mgPOP-1 expression and POP-1 immunostaining. (A–D) Confocal micrograph projections of mgPOP-1 expression in living embryos. (E–H) Single focal plane micrographs of a fixed embryo stained for endogenous POP-1. (A, B) mgPOP-1 levels are higher in MS than in E. Note the punctate appearance of signal in MS. A slightly elevated level of signal is visible in the E cytoplasm as compared to the MS cytoplasm. In (B), the contrast of the image has been digitally enhanced, and the nuclear signal has been deleted, to accentuate this difference. (C) mgPOP-1 levels are higher in MSa and Ea (arrowheads). (D) High mgPOP-1 levels and puncta appear in both A/P sister nuclei in a *wrm-1(RNAi)* embryo (see Table 1). (E–H) A 28-cell-stage embryo stained for endogenous POP-1. Immunostaining shows subnuclear coalescence of signal (arrow), similar to that seen with mgPOP-1 in living embryos. (H) Image from (E) pseudocolored by pixel value to permit discrimination of small level differences by color, as shown by the legend below the montage. Note presence of puncta (red foci). (I–P) Time-lapse sequence of a mgPOP-1-expressing embryo. Time in minutes is indicated for each panel, with $t = 0$ arbitrarily set for (A) Images are pseudocolored as in (H). A digital movie version of this time lapse series is available at http://www.idealibrary.com. (I) A 16-cell-stage embryo with the MS daughters in mid-interphase. Note the presence of bright puncta (red). (J) Immediately before mitosis, nuclear levels in MSa are reduced. (K) Nuclear signal disappears during mitosis of MSa and MSp. (The image stack was acquired just before and during mitosis, resulting in the inclusion of a focal plane with nuclear signal.) (L) Nuclear A/P differences are visible immediately after formation of MS granddaughter nuclei. (This reduction in anterior sisters is more striking in H.) (M) As interphase progresses, the anterior signal becomes more intense. Puncta begin to coalescence (red). (N) In MSaa and MSpa, puncta coalesce into discrete structures (red). In contrast, the nuclear levels in Ea, which have been decreasing steadily during interphase, appear similar to the levels in Ep just prior to mitosis. Note that the E daughter nuclei have ingressed into the interior of the embryo, marking the onset of gastrulation. (O) Prior to mitosis of the MS granddaughters, the nuclear levels begin to drop in MSaa and MSpa. Some weak asymmetry is visible in the four E granddaughters (outlined), which are formed from a cleavage oriented along the left–right, not A/P axis. (P) By mid-interphase, the levels in the E granddaughters are similar (outlined). High levels and puncta are visible in the MS^8 A/P sisters. (Q) Time-lapse series showing formation of mgPOP-1 puncta in an MSpa nucleus. Images from a single focal plane were acquired every 60 s starting immediately after the MSpa nucleus reformed, and ending shortly before prophase. Images are pseudocolored as in (H). The MSpa nucleus is approximately 5 μm in diameter. In (A–P), the eggshell has been indicated with a dotted white line. Sister nuclei are joined with a yellow line in some images. Anterior is to the left and dorsal is up.

TABLE 1
Relative GFP Levels of mgPOP-1 in Various Mutant Backgrounds

Genotype	Gene product	EMS lineage phenotype[a]	Sister blastomeres				
			E/MS	Ea/Ep	MSa/MSp	Exl/Exr[b]	MSxa/MSxp
wild type			A > P	A > P	A > P	A = P	A > P
cbp-1(RNAi)	CBP/p300 homolog	no endoderm (Shi and Mello, 1998)	A > P	A > P	A > P	A > P[c]	A > P
hda-1(RNAi)	histone deacetylase	embryonic arrest (Shi and Mello, 1998)	A > P	A > P	A > P	A = P	A > P
pop-1(zu189)	TCF/LEF	MS → E (Lin *et al.*, 1995)	A > P	A > P	A > P	A = P	A > P
apr-1(RNAi)	APC-related	26% no endoderm (Rocheleau *et al.*, 1997)	A = P[d]	A = P[d]	A = P[d]	A = P[d]	A = P[d]
apr-1(RNAi); mom-2(RNAi)	Wnt (*mom-2*)	100% no endoderm (Rocheleau *et al.*, 1997)	A = P	A = P	A = P	A = P	A = P
bar-1(RNAi)	β-Catenin	none (Eisenmann *et al.*, 1998)	A > P	A > P	A > P	A = P	A > P
lit-1(RNAi)	Nemo-like kinase	E → MS fate change (Rocheleau *et al.*, 1999; Meneghini *et al.*, 1999)	A = P	A = P	A = P	A = P	A = P
lit-1(t1512)[e] [15°C]		no phenotype (Kaletta *et al.*, 1997)	A > P	A > P	A > P	A = P	A > P
lit-1(t1512)[e] [25°C]		E → MS (Kaletta *et al.*, 1997)	A = P	A = P	A = P	A = P	A = P
lit-1(t1534)[e]		2% E → MS (Rocheleau *et al.*, 1999)	A = P	A = P	A = P	A = P	A = P
wrm-1(RNAi)	β-Catenin	E → MS (Rocheleau *et al.*, 1997; Thorpe *et al.*, 1997)	A = P	A = P	A = P	A = P	A = P

Note. A > P, anterior nuclei showed higher levels of signal than posterior nuclei; A = P, nuclear levels were similar; p → a, posterior to anterior; underlined results indicate differences from wild type.

[a] Phenotypes are described for E and MS only. Unless otherwise noted, phenotypes described are 100% penetrant. In the presence of the mgPOP-1 transgene, all mutations or RNAi produced indistinguishable phenotypes from those previously reported.

[b] Relative levels were observed during mid-interphase.

[c] The E daughters in *cbp-1(RNAi)* embryos divide in an anterior–posterior direction on the ventral surface of the embryo (Shi and Mello, 1998).

[d] Levels of nuclear signal were greatly reduced in *apr-1(RNAi)* embryos.

[e] These embryos were also homozygous for *unc-32(e189).*

lacking the entire C terminus, including the HMG box, still show nucleocytoplasmic asymmetry. Collectively, these results suggest that the β-catenin domain, HMG box, and the C terminus of the protein are not required for regulation of this asymmetric behavior; hence, the 124-amino-acid region between the two domains may contain a critical A/P regulatory domain (see Discussion).

POP-1 Undergoes Intranuclear Redistribution in Unsignaled Sister Cells

The finding that overexpressed POP-1 supports normal embryonic development suggests that POP-1 may be qualitatively different in anterior and posterior cells, i.e., that it is not the amount of POP-1 in posterior cells per se that inactivates its repressor function, but rather an alteration in the protein. Observations of the dynamics of mgPOP-1 behavior provided additional evidence for such qualitative differences in POP-1 in anterior and posterior sisters. We found that mgPOP-1 forms prominent puncta specifically in the nuclei of anterior (unsignaled), but never in posterior, sisters (e.g., Fig. 3A). These structures, which do not coincide with nucleoli, are observed in all anterior nuclei of A/P sisters, even at the E^8 stage, when the levels of mgPOP-1 per nucleus have been significantly diluted. Thus, the inability

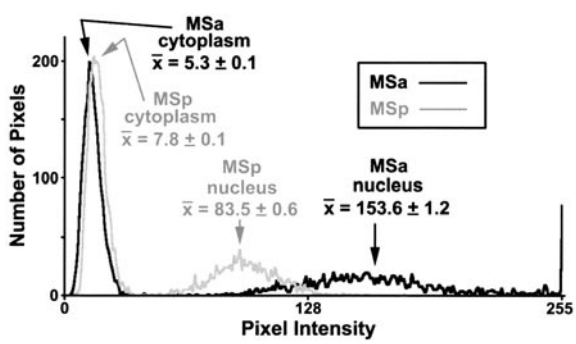

FIG. 4. Quantification of GFP levels for two sister cells expressing mgPOP-1. The histogram plots the number of pixels of a particular signal intensity against intensity value for the sister pair MSa/p. The average nuclear pixel intensities of MSa and MSp give an A:P ratio of ~1.84:1, while the cytoplasmic levels give an A:P ratio of ~0.68:1. The A/P differences in both cases are statistically significant ($P < 10^{-6}$). Given the larger relative size of the cytoplasm, the data suggest that the lower nuclear signal in MSp can be accounted for by the increase in cytoplasmic signal. Signal intensities were quantified by using ImageTool v2.00 (University of Texas Health Science Center, San Antonio, TX) by enclosing areas of interest from confocal micrograph projections. Quantification from individual focal planes, rather than projected stacks, yielded similar results. Average background autofluorescence was subtracted from each data set.

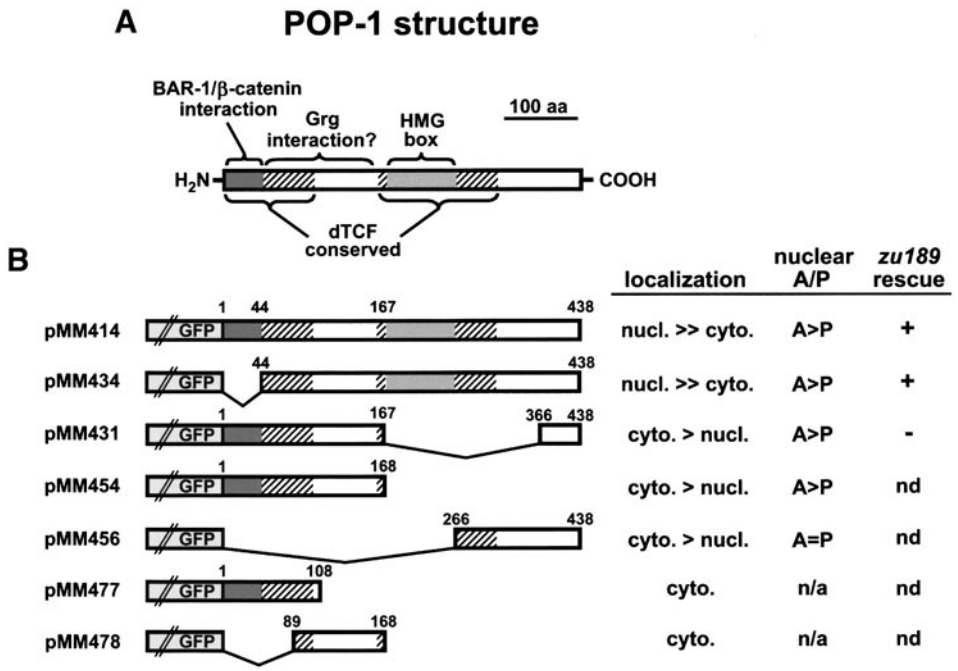

FIG. 5. POP-1 structure and *in vivo* properties of POP-1 domains. (A) Conserved structural features of POP-1. The amino-terminal 44 amino acids are required for interaction with the β-catenin BAR-1 (Korswagen *et al.*, 2000). The central HMG box shares 77% similarity with vertebrate HMG boxes. Two extended regions, which include the β-catenin interaction and HMG box regions, share similarity with *Drosophila* dTCF/Pangolin (46 and 67% similarity, respectively). An extended region shares weak similarity (48% similarity with several gaps) to the Groucho-related gene (Grg) interaction domain of XTcf-3 (Brantjes *et al.*, 2001). (B) Expression of altered POP-1 transgenes and rescue of *pop-1(zu189)* mutants. Plasmid names, diagrams of GFP::POP-1 fusions, and amino acid positions at protein fusion junctions are shown. Expression was assayed in at least two independent lines in each case. Abbreviations: nucl., nuclear levels; cyto., cytoplasmic levels; n/a., not applicable; nd, not determined.

to detect these structures in nuclei of posterior cells is not simply attributable to the twofold lower nuclear signal compared with that in their sisters. Moreover, these structures are likely to reflect a *bona fide* property of POP-1 in a Wnt-unmodified state, rather than a feature of the mgPOP-1 construct per se: similar puncta are also observed with myc epitope-tagged POP-1 and by immunostaining of endogenous POP-1 in wild-type embryos (Figs. 3E–3H; and data not shown) but are not observed with a similarly overexpressed GFP::MED-1 transgene (mgMED-1). Time-lapse studies reveal that these anterior-specific puncta undergo dynamic changes during interphase. They are initially visible as many small speckles in each nucleus, which progressively coalesce into larger structures by mid-interphase (see time series in Fig. 3Q) and condense to as few as two large domains by late interphase. Thus, intracellular and intranuclear POP-1 distributes very differently in Wnt/MAPK-signaled and unsignaled cells, further substantiating the notion that the protein is qualitatively different in anterior and posterior sisters.

We investigated the requirements for the mgPOP-1 anterior-specific puncta. In *Drosophila,* the CBP/p300 homolog dCBP is known to antagonize Wnt signaling by lowering the affinity of the POP-1 homolog dTCF for Armadillo/β-catenin through acetylation of dTCF (Waltzer and Bienz, 1998). Depletion of *cbp-1,* a CBP/p300 homolog that is required for *end-1, -3* activation (Calvo *et al.*, 2001), had no effect on the appearance of mgPOP-1 asymmetry or puncta. In *Xenopus,* the C-terminal binding protein (CtBP) binds to XTcf-4 and functions as a corepressor (Brannon *et al.*, 1999); we did not observe any change in mgPOP-1 in embryos in which the *C. elegans* CtBP was depleted by RNAi (not shown). PSA-4, a SWI/SNF component homolog, is required for LIN-44/Wnt-regulated asymmetric division of the postembryonic T cell (Herman *et al.*, 1995, 2001; Sawa *et al.*, 2000). Although *psa-4(RNAi)* results in embryonic lethality (consistent with mutation data; Sawa *et al.*, 2000), we observed no effect on mgPOP-1 distribution other than delayed expression (data not shown). Histone acetylation is a marker for broad chromatin domains (Turner *et al.*, 1992; Forsberg and Bresnick, 2001). Staining with antisera to various histone H4 acetylation states (see Materials and Methods) failed to reveal enrichment of specific acetylation states with the mgPOP-1 puncta. However, elimination of Wnt/MAPK signaling (i.e., by elimination of WRM-1/β-catenin; not shown) results in the appearance of puncta in

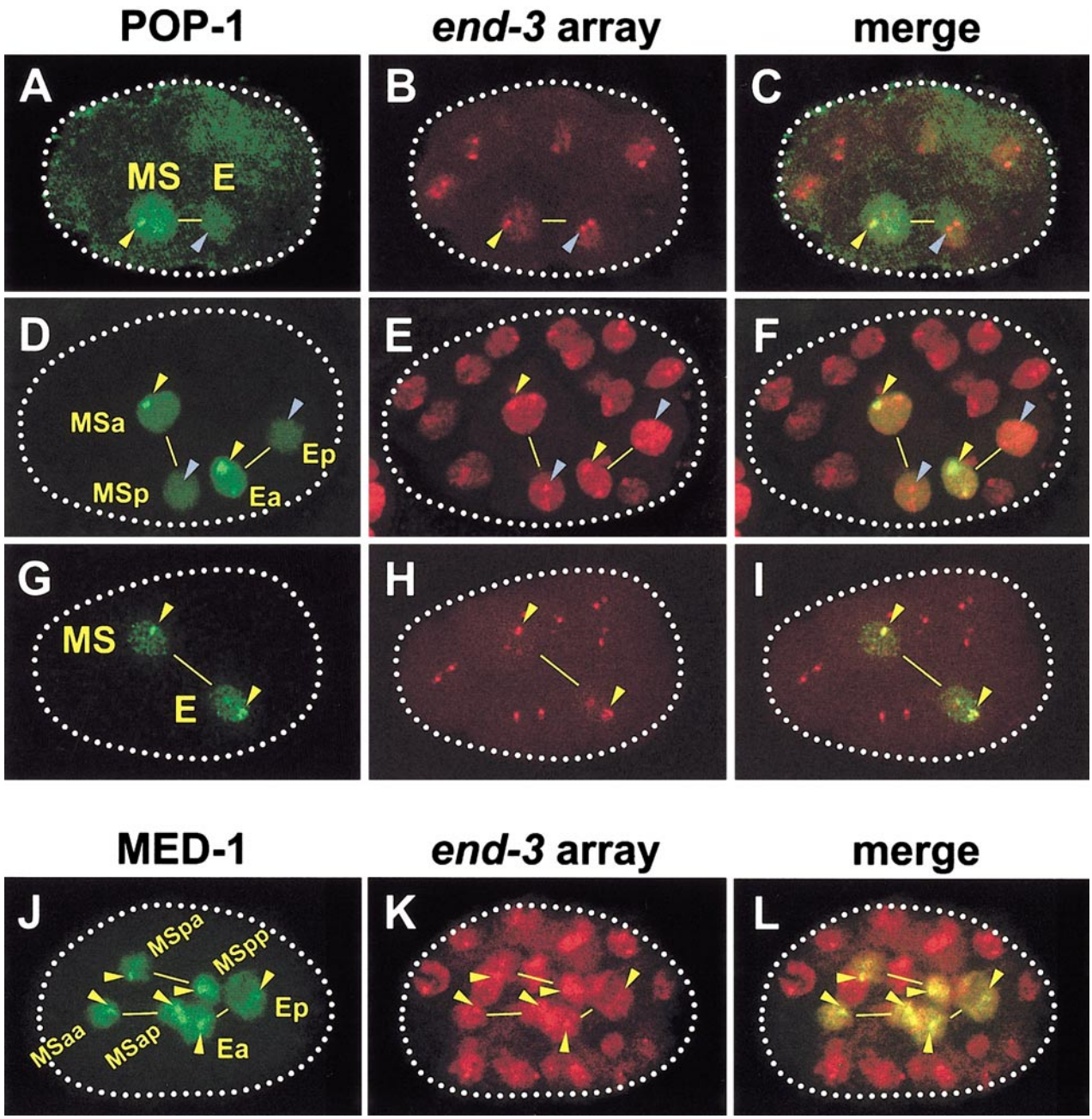

FIG. 6. Interactions of mgPOP-1 and mgMED-1 with the *end-3* promoter. Confocal micrographs show anti-GFP (left column), anti-myc (middle column), and merged images (right column) in fixed embryos expressing either mgPOP-1 or mgMED-1, and an extrachromosomal array carrying the *end-3* target. The *end-3* array also carries multimeric *lac*O sequences, which were detected by their interaction with myc-tagged LacI protein (Belmont and Straight, 1998). Similar results were obtained with an *end-1* target array (not shown). (A–F) Colocalization of mgPOP-1 with the *end-3* array is observed in the anterior daughters MS, MSa, and Ea (yellow arrowheads) but not the posterior daughters E, Ep, and MSp (blue arrowheads). (G–I) In a *wrm-1(RNAi)* background, mgPOP-1 colocalizes with the *end-3* array in both daughters of an A/P division, such as in E and MS shown here (yellow arrowheads), and in both daughters of E and MS (not shown). (J–L) While the mgPOP-1 repressor localizes to the *end-3* promoter in anterior nuclei only, the mgMED-1 activator interacts with the *end-3* promoter in both anterior and posterior daughters (yellow arrowheads). Similar MED-1 foci have also been observed with control arrays containing *end-1* or *end-3* promoter sequences, independent of transgene marker DNA or the *lacI/lacO* plasmids, and also in MS and E in living embryos (data not shown). We interpret these results to mean that POP-1 and MED-1 can both interact with the *end-3* promoter, and that this double interaction results in repression of *end-3* in MS. Anterior is to the left, and sister nuclei are connected by a line.

posterior sister nuclei. Thus, the Wnt/MAPK abolishes the formation of these intranuclear domains presumably by altering POP-1.

The end-1, -3 Genes Are in Vivo Targets of POP-1 in MS

Although MED-1 is a potent activator of *end-1, -3* in the E lineage (Maduro *et al.,* 2001), its presence in the MS lineage does not result in *end* transgene activation, presumably because POP-1 in its repressive state either directly or indirectly blocks MED-1 activity. We investigated the possible direct interaction of POP-1 with *end-1, -3* in early embryos by examining their *in vivo* interactions in whole animals (Carmi *et al.,* 1998; Fukushige *et al.,* 1999; Materials and Methods). The multi-copy nature of *C. elegans* transgenes allows detection of such protein–DNA interactions owing to the high copy number of target sequences present in extrachromosomal transgenic arrays. Transgenic extrachromosomal arrays carrying either the *end-1* or *end-3* promoters were used to probe association with mgPOP-1. As anticipated by the requirement for POP-1 in repression of endoderm fate in MS, but not E, we observed interaction of mgPOP-1 with *end-1* and *end-3* specifically in the MS blastomere, as evidenced by an intranuclear spot of immunoreactive GFP (from mgPOP-1) corresponding to the location of the *end*-bearing array (Figs. 6A–6C). While the failure to observe an interaction in E might be attributable to the approximately twofold lower nuclear level of mgPOP-1 in E vs MS (see below), such a difference is unlikely to preclude detection of a subnuclear spot in which the signal has been concentrated. mgPOP-1 did not associate with arrays containing control genes, including the *elt-2* promoter (a target of END-1, -3), or the array expressing mgPOP-1 itself (data not shown). The *end-1* and *end-3* promoters contain numerous (A/T)(A/T)CAAAG TCF-1 consensus sites (Oosterwegel *et al.,* 1991; J. Kasmir, M.F.M. and J.H.R., unpublished observations), and POP-1 can bind an optimized TCF-1 binding site in cultured cells and *in vitro* (Korswagen *et al.,* 2000). These observations show that *end-1* and *end-3* are direct targets of POP-1 in MS.

We further observed that mgPOP-1 associates with the *end* arrays in MSa and Ea, the anterior daughters of MS and E (Figs. 6D–6F). This was somewhat unexpected since the *ends* are not expressed in the MSp lineage (in which no interaction is detected) and there is no apparent repression in the Ea lineage. These data show that an A/P asymmetric POP-1–target interaction can occur reiteratively, suggesting that a mechanism exists to restrict the output of this interaction to the appropriate stage within a lineage (see Discussion).

To examine the relevance of the Wnt/MAPK signaling pathway on the mgPOP-1/*end* interaction, we analyzed embryos depleted for WRM-1/β-catenin, which is essential for POP-1-dependent repression of endoderm fate (Rocheleau *et al.,* 1997; Thorpe *et al.,* 1997). In such embryos, mgPOP-1 was found to associate with the *end*-bearing arrays in both MS and E, as well as in their daughters (Figs. 5G–5I; and data not shown). Thus, Wnt/MAPK signaling inhibits the interaction of mgPOP-1 with *end-1, -3* in the nuclei of posterior sisters, explaining the mechanism by which POP-1-mediated repression of the *end* genes, and endoderm fate, occurs in the E cell.

POP-1 in Its Repressive State Prevents Activation by end-3-Bound MED-1 in MS

How does POP-1 repress transcriptional activation of *end-1, -3* in the MS lineage? In *pop-1(−)* embryos, MS expresses *end-1, -3* and adopts an E fate in a *med*-dependent manner (Maduro *et al.,* 2001; Calvo *et al.,* 2001; J. Zhu, M.F.M. and J.H.R., unpublished observations). MED-1 (and its homolog MED-2) is a GATA factor, and there are several GATA recognition sequences in the *end-1* and *end-3* promoters, suggesting that MED-1/2 may activate *end-1, -3* directly (consensus HGATAR; Lowry and Atchley, 2000; J. Kasmir, M.F.M. and J.H.R., unpublished observations). Given that POP-1 apparently binds *end-1, -3* when functioning as a repressor, one plausible mechanism might be that it precludes binding of MED-1, -2 in MS. To examine this possibility, we tested the ability of GFP-tagged MED-1, expressed under its own promoter (mgMED-1) to bind *end-1* or *end-3* transgenes (see Materials and Methods). The GFP tag did not discernibly alter MED-1 function, as the same GFP::MED-1 chimera driven by a heat shock promoter was able to *trans*-activate *end-3* expression and generate ectopic endoderm, similar to untagged MED-1 (Maduro *et al.,* 2001; data not shown). We observed that mgMED-1 produces intranuclear spots in all early EMS descendants when *end-1* or *end-3* were present on an extrachromosomal array (Fig. 6J), but not in embryos lacking such an array, indicating that mgMED-1 binds to *end-1* and *end-3* in both the MS and E lineages, in both anterior and posterior nuclei. The nuclear spots seen in these experiments represent interaction of mgMED-1 with the *end*-containing array (Figs. 6K and 6L). We conclude that GFP::MED-1 interacts with the *end-1* and *end-3* promoters in both the E and MS lineages *in vivo,* indicating that POP-1 must repress activation of the *ends* by a mechanism that does not preclude simultaneous binding of the MEDs. Although the assay used generates subnuclear spots, we do not know whether the puncta exhibited by anterior-specific mgPOP-1 in living embryos (in the absence of *end* target arrays) represent similar association of POP-1 with repressive targets.

DISCUSSION

We report the first observation in an intact embryo of the dynamic behavior of a Lef factor, and its interactions with its target, as it responds to Wnt signaling. The major advances from these studies are: (1) "POP-1 asymmetry" (Lin *et al.,* 1998) is the result of the rapid nucleocytoplasmic redistribution of POP-1 by a Wnt/MAPK-dependent mecha-

nism occurring immediately after a cell is born, rather than differential stability, synthesis, or immunoreactivity. (2). While an internal 124-amino-acid segment is apparently required for the asymmetric nucleocytoplasmic distribution of POP-1, the amino-terminal β-catenin interaction domain, the HMG box DNA-binding domain, and the C terminus are dispensable for this event. (3) Based on its *in vivo* behavior, POP-1 appears to be qualitatively different in Wnt/MAPK-signaled and unsignaled cells. It undergoes an intranuclear rearrangement in the latter, progressively coalescencing into subnuclear domains. (4) The *end-1* and *end-3* genes are the first direct POP-1 targets identified and the apparent qualitative difference in POP-1 is correlated with its ability to bind these targets in unsignaled cells. (5) When it functions as a repressor, both POP-1, and an activator, MED-1, can bind to the *end-1* and *-3* targets; thus, POP-1 repressor does not preclude binding of an activator, but instead functions at a post-binding step to block transactivation. This dynamic behavior of POP-1 may reflect a global mechanism by which it functions as a reiterative developmental switch that generates nonequivalent sister cells throughout *C. elegans* development.

POP-1 Asymmetry and Endoderm Specification

The TCF/LEF protein POP-1 is the terminal regulator of a recursive Wnt/MAPK-dependent switch that operates throughout *C. elegans* development in many asymmetric A/P divisions (Lin *et al.,* 1998). We find that mgPOP-1 can interact with extrachromosomal arrays containing the *end* promoters in the nucleus of MS, the blastomere in which POP-1 function is required to prevent *end-1, -3* activation and endoderm specification (Fig. 7A). In the E nucleus, this interaction is not detectable. This pattern of POP-1/*end-3* interaction is recapitulated in the E and MS daughter cells, such that POP-1 interacts with the *end* promoters in MSa and Ea. These latter interactions appear to be gratuitous, as POP-1 function is not required to prevent inappropriate activation of *end-1, -3* in these cells; however, differential POP-1 activity in Ea and Ep is apparently required to pattern expression of genes in the later endoderm (Schroeder and McGhee, 1998). Our results therefore support the notion that endoderm specification results from the *combination* of direct positive input by the lineage-specific factors MED-1, -2 (in MS and E) and direct negative input by the POP-1 switching system (in MS) (Fig. 7A). The P_2–EMS interaction results in a change in POP-1, which prevents the interaction between POP-1 and *end-1, -3* in the E nucleus, permitting MED-1, -2 to activate *end-1, -3* and specify an E fate.

Why, then, are nuclear levels of POP-1 reduced by Wnt/MAPK signaling? WRM-1/β-catenin and LIT-1/NLK are both required for endoderm fate and POP-1 asymmetry (Rocheleau *et al.,* 1997; Thorpe *et al.,* 1997; Shin *et al.,* 1999). The WRM-1/LIT-1 kinase can phosphorylate POP-1, which has been proposed to cause POP-1 degradation (Shin *et al.,* 1999; Rocheleau *et al.,* 1999). Based on several observations, degradation appears not to be the mechanism for generating POP-1 asymmetry. First, when a posterior cell divides, it produces an anterior daughter that expresses similar levels of mgPOP-1 as the anterior daughter of its sister. This occurs even in the absence of new protein synthesis, as transcripts for mg*pop-1* are no longer detectable by the 16-cell stage, but asymmetry recurs for many cell generations thereafter. A/P sister cells must therefore contribute a similar amount of total POP-1 protein to each daughter. This is consistent with our observation that the elevated cytoplasmic level of mgPOP-1 is similar between mitotic A/P sisters. Second, measurement of GFP signals in the cytoplasms of E, MSp, and Ep revealed elevated levels that can account for the A/P nuclear mgPOP-1 differences. Our analysis of mgPOP-1 dynamics, therefore, indicates that Wnt/MAPK signaling causes POP-1 asymmetry via nucleocytoplasmic redistribution. This is consistent with previous findings in cultured cells, in which a slight increase in the proportion of cells with cytoplasmic myc-POP-1 was observed when myc-POP-1 was coexpressed with WRM-1/LIT-1 (Rocheleau *et al.,* 1999).

The initial observation that POP-1 levels are lower in E than in MS, coupled with the loss of asymmetry and E specification seen in Wnt/MAPK mutants, led to the proposal that low levels of POP-1 are a requirement for E fate (Lin *et al.,* 1995, 1998; Rocheleau *et al.,* 1997, 1999; Thorpe *et al.,* 1997). However, our results indicate that Wnt/MAPK-induced relief of repression by POP-1 is not the result of down-regulation of POP-1 amounts per se, but rather appears to be the result of a qualitative change in POP-1, which alters its activity. We have found that functional mgPOP-1 or myc-tagged POP-1 can be stably expressed at levels ~10-fold higher than endogenous POP-1 in the early *C. elegans* embryo without causing developmental defects. Comparison of anti-POP-1 signal in the mgPOP-1 strain has shown that there is at least twice as much mgPOP-1 in posterior EMS descendants than in anterior cells in other lineages. Similarly, we have found that overexpression of POP-1 from a heat-shock promoter-driven construct does not result in repression of endoderm development (M.F.M. and J.H.R., unpublished observation). Despite this excess of POP-1, both asymmetry and correct specification of endoderm still occur in the presence of these transgenes. Moreover, we have found that the unusual *lit-1(t1534)* mutant in which POP-1 asymmetry is eliminated nonetheless produces endoderm. These data suggest that POP-1 asymmetry can be uncoupled from its function as a repressor. Therefore, absolute POP-1 levels are not the critical element of POP-1 function in MS/E specification. We propose that the qualitative difference in POP-1 between MS and E, reflected in the interaction with the *end-1, -3* promoters, is the crucial parameter of POP-1 asymmetry that regulates E specification. It is not clear whether this is true for postembryonic A/P asymmetries that require POP-1 function. For example, expression of POP-1 under heat shock control in larval males causes a spicule defect similar to loss-of-function of the HMG-1/2

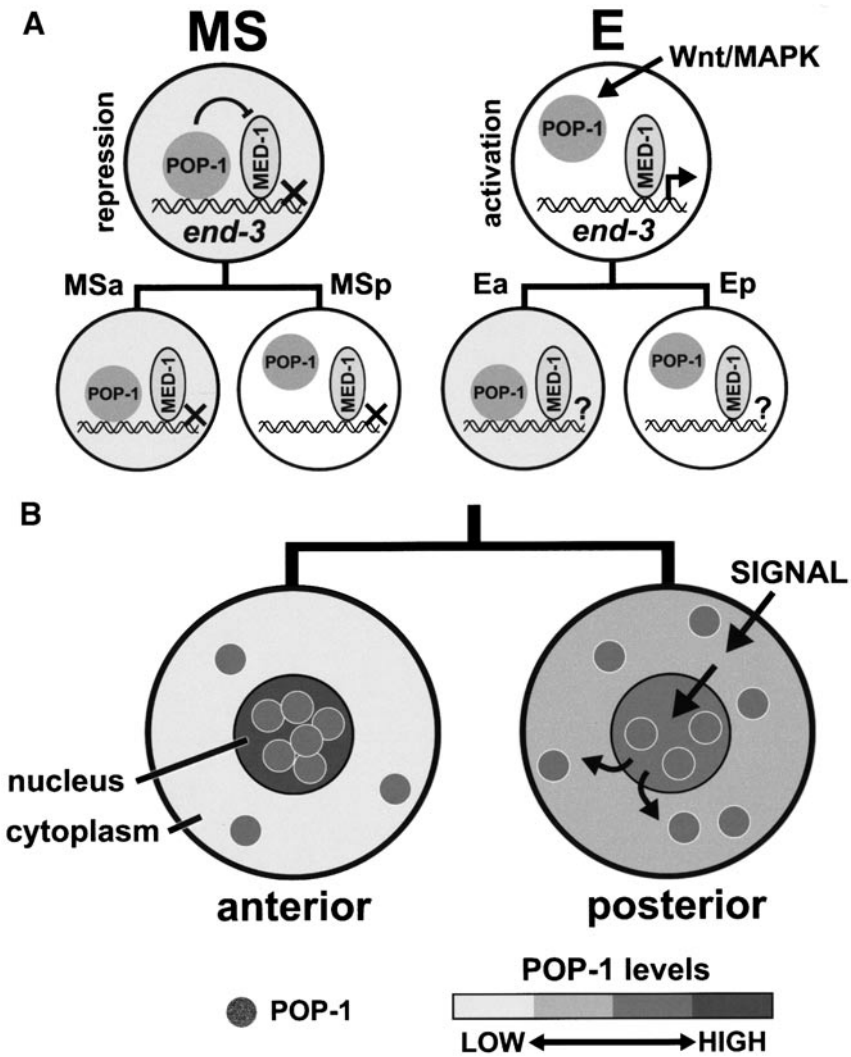

FIG. 7. E specification and POP-1 asymmetry. (A) A model showing the developmental switch that restricts activation of *end-1, -3* to the posterior daughter of the asymmetrically dividing EMS blastomere. The nuclei of MS, E, and their daughters are shown, with shading to depict higher POP-1 levels. In the MS nucleus, both POP-1 and MED-1 interact with the *end-3* promoter, where POP-1 prevents transcriptional activation of *end-3* by MED-1. In E, this repression is relieved by the reduced affinity of POP-1 for *end-3*. In the MS daughters, the pattern of MED-1/POP-1 interaction is recapitulated, but *end* activation does not occur. The pattern is also repeated in Ea/Ep; however, while detectable *end* mRNA and reporter gene expression levels appear to be similar, it is not known whether the *end* genes are actively transcribed or repressed at this stage. Although only shown for MED-1 and *end-3*, these interactions presumably occur among their redundant homologs (i.e., between MED-1/MED-2 and *end-1/end-3*). (B) A model for the generation of POP-1 asymmetry based on *in vivo* mgPOP-1 behavior. In anterior cells, POP-1 (circles) is present at high nuclear levels, and low (but significant) cytoplasmic levels. In posterior cells, a signaling event, transduced through Wnt/MAPK components, results in a redistribution of POP-1. Nuclear levels of POP-1 are reduced by approximately 50% in signaled cells, perhaps by rapid export from the nucleus (Rocheleau *et al.*, 1999) shortly after mitosis is complete; alternatively, POP-1 in a posterior (signaled) cell may be imported into the newly formed posterior nucleus less efficiently than in its anterior sister. The remaining POP-1 accumulates in the cytoplasm, conserving the total (nuclear + cytoplasmic) levels of POP-1 in each sister cell.

homolog SON-1 (Jiang and Sternberg, 2000). In addition, while expression of POP-1 in hermaphrodite larvae caused no effect on the asymmetric division of the T neuroblast, migration defects in the daughters of the QL neuroblast (similar to Wnt pathway mutants) were seen (Herman, 2001). These observations suggest that absolute POP-1 levels may be important for some, but not all, postembryonic A/P asymmetries.

That there is a marked qualitative difference in POP-1 between A/P sisters is further evidenced by our finding that

mgPOP-1 (and immunoreactive POP-1) undergoes a striking intranuclear relocalization during early interphase of a newly formed anterior (unsignaled) cell that is never seen in posterior cells. This progressive coalescence results in association of most of the nuclear protein with as few as two domains within all anterior sisters of A/P divisions. The presence of these intranuclear regions of POP-1 localization in unsignaled cells correlates with the repressive activity of POP-1, its higher nuclear: cytoplasmic distribution, and its ability to associate with the *end-1, -3* target genes. While it is unclear whether these events are all causally related, these observations raise the possibility that POP-1 in its repressive form may associate with, or even promote, the formation of extended nuclear domains involved in transcriptional repression. In this regard, it is of interest that, in both *C. elegans* and a related nematode, *C. briggsae,* two of its targets, *end-1* and *end-3,* are in close proximity, though not adjacent in the genome, possibly suggesting a mechanism for coordinate regulation. Whatever the precise mechanism of repression that POP-1 uses, it does not appear to act by excluding binding of activators, since the MED-1 activator of *end-1, -3* is bound even in cells (MS lineage) in which these genes are fully repressed.

POP-1 Repressive Function and Asymmetric Cell Division

Why has a similar reiterative role for TCF/LEF proteins in asymmetric cell division not been observed in other systems? One possibility is that this is a unique adaptation in *C. elegans,* arising from the rapid cell cycles that occur in early embryogenesis. We have found that expression of GFP-tagged dTcf (i.e., the *Drosophila* TCF homologue, also known as Pangolin) in the EMS lineage of *C. elegans* results in weak nuclear localization which is A/P symmetric (our unpublished results), suggesting that the *Drosophila* protein lacks structural elements associated with POP-1 asymmetry.

Recently, it has been demonstrated that TCF/LEF proteins can act as repressors in the absence of signaling (Kim *et al.,* 2000; Merrill *et al.,* 2001). Unlike bipartite β-catenin-TCF activators, repressive TCFs function in the absence of β-catenin: truncated TCFs lacking the conserved amino-terminal β-catenin interaction domain can still function as repressors by interacting with corepressors of the Grg (Groucho-related gene) family (Kim *et al.,* 2000; Brantjes *et al.,* 2001). This interaction has been mapped to a Grg domain located between the β-catenin and HMG box regions of vertebrate TCFs (Brantjes *et al.,* 2001). POP-1 contains a region with 27% identity to a vertebrate Grg domain in this same region, and POP-1 associates with the Groucho-like protein UNC-37, which is proposed to act in MS to repress endoderm fate (Fig. 5A; Roose *et al.,* 1998; Brantjes *et al.,* 2001; Pukrop *et al.,* 2001; Calvo *et al.,* 2001). Consistent with a conserved mechanism for POP-1/TCF repressive function, we have found that POP-1 lacking the β-catenin interaction domain can rescue MS fate in *pop-1* mutants. Moreover, all deleted mgPOP-1 constructs that retain the putative Grg domain still demonstrate some nuclear asymmetry. An intriguing possibility, therefore, is that both asymmetry and repressive function of POP-1 are regulated via this domain.

Temporal Restriction of Wnt/MAPK Responsiveness

The recursive nature of the POP-1 anterior–posterior switching system suggests that activation of genes responsive to this mechanism must be restricted in their potential to respond, such that POP-1 activity influences transcription only at the time the A/P decision is made (Lin *et al.,* 1998). We have observed the interaction of mgPOP-1 with *end-1, -3* in MS at the time the E/MS decision is made, but also in the anterior daughters of MS and E, cells in which the POP-1 switching system does not contribute to *end* promoter activity. Thus, it may be that POP-1 can initiate a repressive state only at the time that transcription of a gene is first established (e.g., *end-3* in the E cell), but is not subsequently required to maintain this repressed state. Alternatively, the *end* genes may lose their responsiveness to POP-1 and MED-1, -2 after E and MS have been specified. Indeed, nuclear accumulation of *end-3* transgene transcripts (evidence for active transcription; Seydoux and Fire, 1995) is restricted to the eight-cell stage (data not shown). In *Xenopus,* a temporal restriction of gene responsiveness is known to occur for the Wnt target genes *siamois* and *Xnr-3,* which become unresponsive to Wnt signal transduction after gastrulation (Darken and Wilson, 2001). These data suggest that a mechanism to temporally restrict target gene responsiveness downstream of Wnt signaling has been evolutionarily conserved. Whether this involves recruitment of repressors, modification of the activators, or a change in chromatin state remains to be examined.

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