β-catenin-driven binary cell fate decisions in animal development

Vincent Bertrand*

The Wnt/β-catenin pathway plays key roles during animal development. In several species, β-catenin is used in a reiterative manner to regulate cell fate diversification between daughter cells following division. This binary cell fate specification mechanism has been observed in animals that belong to very diverse phyla: the nematode *Caenorhabditis elegans*, the annelid *Platynereis*, and the ascidian *Ciona*. It may also play a role in the regulation of several stem cell lineages in vertebrates. While the molecular mechanism behind this binary cell fate switch is not fully understood, it appears that both secreted Wnt ligands and asymmetric cortical factors contribute to the generation of the difference in nuclear β-catenin levels between daughter cells. β-Catenin then cooperates with lineage specific transcription factors to induce the expression of novel sets of transcription factors at each round of divisions, thereby diversifying cell fate. © 2016 The Authors. *WIREs Developmental Biology* published by Wiley Periodicals, Inc.

INTRODUCTION

During animal development a high diversity of cells with different fates is generated from a single egg cell. The formation of distinct cell types involves the combined action of several signal transduction pathways. One such signaling cascade, the Wnt/β-catenin pathway (or canonical Wnt pathway) plays key roles during animal development. It also plays important roles in tissue homeostasis and its misregulation leads to diseases in human such as cancer or congenital malformations.[1,2]

The key transcriptional effectors of this pathway are transcription factors of the T-cell factor (TCF) family and the transcriptional coactivator β-catenin (Figure 1). In general, this pathway is activated by secreted proteins of the Wnt family, as follows. In the absence of Wnt, β-catenin is degraded in the cytoplasm by a destruction complex. This

complex is composed of two scaffolding proteins, Axin and adenomatous polyposis coli (APC), as well as two kinases, casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3). This complex phosphorylates β-catenin, which is then degraded by the proteasome. In the absence of β-catenin, TCF acts as a repressor on Wnt target genes. When Wnt ligands bind their transmembrane receptor Frizzled, Frizzled inhibits the activity of the destruction complex via the cytoplasmic protein Dishevelled. β-Catenin accumulates in the cytoplasm and enters the nucleus, where it binds TCF and activates the transcription of Wnt target genes.

The Wnt/β-catenin pathway is present in all animals from sponges to human. Studies of its function in various animals have revealed some conserved roles during animal development. Perhaps the most striking feature is the key role played by this pathway in the specification of the primary axis in many animals (anteroposterior and/or animal–vegetal axis)[3] (Figure 2). Wnt promotes posterior identity and Wnt ligands are preferentially expressed in the posterior region in many bilaterians including vertebrates, cephalochordates, planarians, or nematodes. In addition, the Wnt/β-catenin pathway also plays a role in

*Correspondence to: vincent.bertrand@univ-amu.fr

Aix-Marseille Université, CNRS, Institut de Biologie du Développement de Marseille, Marseille, France

the specification of the primary axis in cnidarians suggesting that this function predates the emergence of bilaterians.

In this review, I discuss another developmental function of the Wnt/β-catenin pathway that recently emerged as being shared between distant animal phyla:[4] the reiterative use of β-catenin mediated binary switches to diversify cell fates. I first describe the different contexts where this system has been shown to operate (nematodes, annelids, and ascidians) and discuss its potential implication in vertebrate stem cell lineages. I then analyze how these β-catenin asymmetries are generated and how they are integrated into gene regulatory networks to generate cell fate diversity.

REITERATIVE β-CATENIN ASYMMETRIES DRIVING CELL FATE SPECIFICATION IN DIVERSE ANIMAL PHYLA

The use of reiterative β-catenin-mediated binary switches during animal development was first observed in the nematode *Caenorhabditis elegans*. The *C. elegans* embryo develops with a fixed cell lineage and many cells are generated by a succession of asymmetric divisions oriented along the anteroposterior axis.[5] Gene loss of function experiments at specific time points, using a temperature-sensitive mutant, combined with lineage analysis revealed that
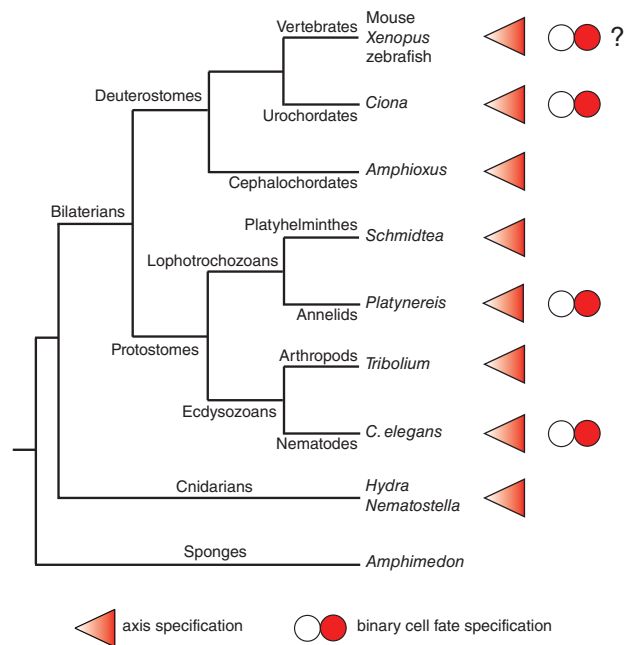

FIGURE 2 | Role of β-catenin in axis specification and reiterative binary cell fate specification in metazoans. Phylogenetic tree summarizing the role of Wnt signaling in axis specification or binary cell fate specification, as indicated by the key. The question mark for binary cell fate specification in vertebrates illustrates the potential role of β-catenin in several vertebrate stem cell lineages (see text for details).

many of these anteroposterior divisions are regulated by a common genetic pathway that generates different identities in the anterior and posterior daughters of each successive division.[6] Further studies have revealed that this pathway is a variant of the Wnt/β-catenin cascade named the Wnt/β-catenin asymmetry pathway.[7] The core of this pathway is formed by two β-catenins (WRM-1 and SYS-1)[8,9] and a TCF transcription factor (POP-1).[10] SYS-1/β-catenin acts as a coactivator for POP-1/TCF, as do classic β-catenins,[9] while WRM-1/β-catenin regulates POP-1/TCF nuclear localization.[8,11,12] Following many anteroposterior asymmetric divisions, the β-catenins WRM-1 and SYS-1 accumulate in the nucleus of the posterior daughter cell but not in the nucleus of the anterior daughter (Figure 3(a)).[13–17] The TCF transcription factor POP-1 is also asymmetric but in an opposite way: POP-1/TCF nuclear levels are higher in the anterior daughter cell than in the posterior daughter.[10,11,17] As a consequence, there is a high SYS-1/β-catenin to POP-1/TCF ratio in the posterior nucleus, POP-1 is mostly bound to SYS-1 and acts as a transcriptional activator.[9] In the anterior nucleus, there is a low SYS-1 to POP-1 ratio, POP-1 is mostly free of SYS-1 and acts as a transcriptional repressor.[9]
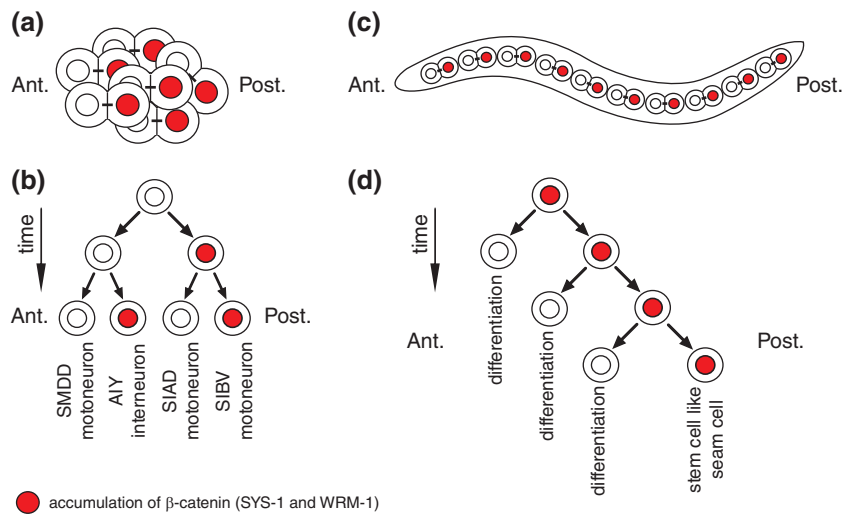
FIGURE 3 | Reiterative β-catenin asymmetries during *C. elegans* development. (a) Early embryo of *C. elegans*. (b) Example of a terminal neuronal lineage in the late embryo. (c) Early larva of *C. elegans*. The stem cell-like seam cells are located on each side of the larva. The seam cells are presented just after one of their divisions, the anterior daughter 'β-catenin OFF' will differentiate, while the posterior daughter 'β-catenin ON' will remain a seam cell. (d) Typical succession of divisions in a seam cell lineage of the *C. elegans* larva. Ant., anterior; Post., posterior.

Figure 3(b) illustrates the reiterative use of this pathway in an embryonic neuronal lineage leading to a diversification of neuronal cell fates.[18,19]

This pathway also regulates many asymmetric divisions during *C. elegans* larval development. One particularly interesting example is the stem cell-like asymmetric divisions of the seam cells (Figure 3(c)). Seam cells are located on each side of the larva and undergo a succession of asymmetric divisions oriented along the anteroposterior axis.[20] Following asymmetric division, the anterior daughter usually differentiates into an epidermal or neural cell, while the posterior daughter retains a seam cell fate and keeps dividing (Figure 3(d)). Following each successive asymmetric division, the β-catenins WRM-1 and SYS-1 accumulate in the nucleus of the posterior daughter, which retains a seam cell fate, while POP-1 is enriched in the nucleus of the anterior daughter, which differentiates.[11,14,15,21,22] In addition, experimental manipulations of the Wnt/β-catenin asymmetry pathway demonstrated that this pathway regulates the fates of the daughter cells in a reiterative manner promoting the seam cell (posterior) fate over the differentiated (anterior) fate at each successive division.[14,21,23] Therefore, reiterative β-catenin-mediated cell fate switches play key roles during both embryonic and larval development in *C. elegans*.

A reiterative use of β-catenin-mediated binary cell fate decisions has also been observed more recently in the annelid *Platynereis dumerilii*.[24] The embryos of *Platynereis* develop with a succession of asymmetric divisions oriented along the animal–vegetal axis.[25] *Platynereis* has only one *β-catenin* gene and following most of these animal–vegetal oriented asymmetric divisions, the β-catenin protein accumulates in the nucleus of the vegetal but not the animal daughter cell (Figure 4).[24] In addition, manipulating β-catenin levels at precise time points using drugs revealed that β-catenin promotes vegetal daughter fate over animal daughter fate in a reiterative manner at each successive division.[24] Therefore, reiterative β-catenin switches appear to play a major role in cell fate diversification in the embryo of *Platynereis*, a process remarkably similar to the one observed in the embryo of *C. elegans*.

Successive β-catenin binary fate decisions have also been recently observed in the embryo of a chordate, the ascidian *Ciona intestinalis*, whose genome contains only one *β-catenin* gene.[26] Ascidian embryos develop with an invariant lineage and germ layers are segregated by cell divisions oriented along the animal–vegetal axis (Figure 5(a)).[27] In *Ciona*, at the transition from 4 to 8 cells, a first division oriented along the animal–vegetal axis segregates the ectoderm (animal daughters) from the mesendoderm (vegetal daughters). After this division, β-catenin activity is present in the vegetal daughters but not the animal daughters and promotes mesendoderm fate over ectoderm fate.[26,28–30] At the transition from 16 to 32 cells, a second division oriented along the animal–vegetal axis segregates the margin (mesoderm + some neural) on the animal side from the endoderm on the vegetal side. Following division, β-catenin accumulates in the nucleus of the vegetal
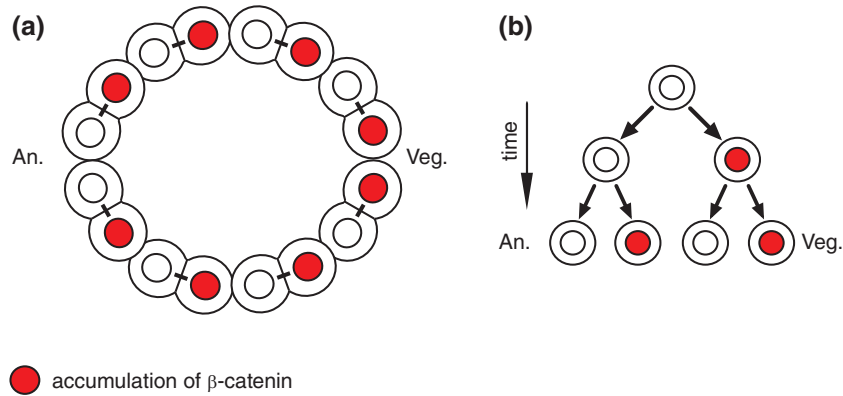
FIGURE 4 | Reiterative β-catenin asymmetries during *Platynereis* development. (a) Early embryo of *Platynereis*. (b) Typical succession of divisions in the early embryo of *Platynereis*. An., animal; Veg., vegetal.
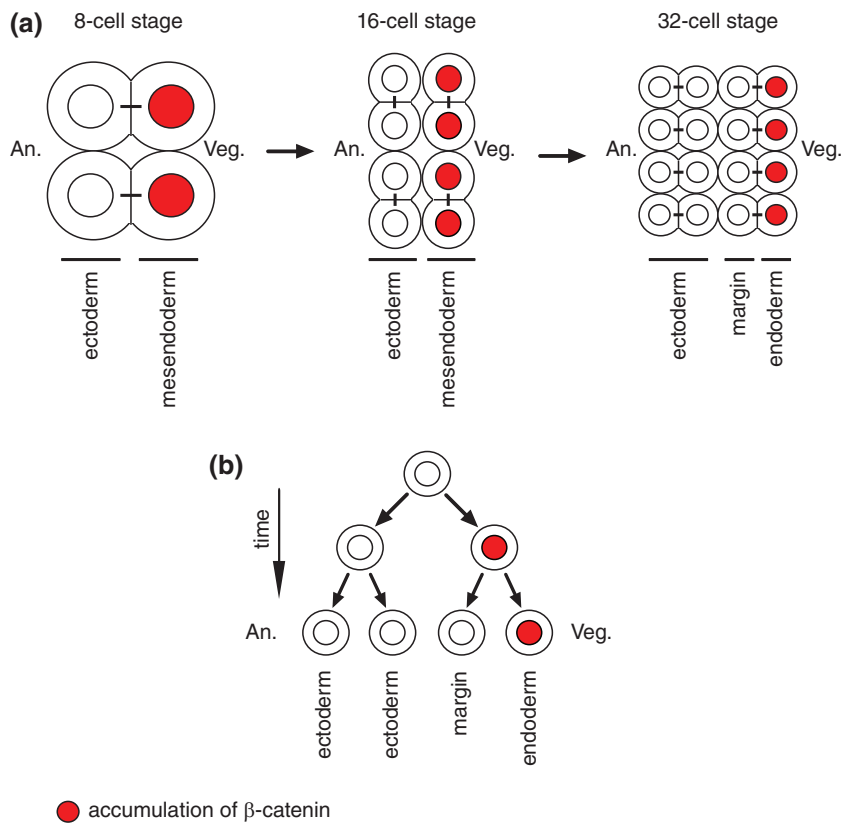

FIGURE 5 | Reiterative β-catenin asymmetries during *Ciona* development. (a) Succession of divisions in the *Ciona* embryo from the 8-cell stage to the 32-cell stage. Only cells of the anterior half of the embryo are presented ('a' and 'A' lineages). (b) Schematic representation of the binary decisions. Only divisions along the animal (an.)–vegetal (veg.) axis are presented (the 8- to 16-cell stage division that is perpendicular to the animal–vegetal axis is omitted).

daughters (but not the animal daughters) and promotes endoderm fate over margin fate.[26] Therefore, two β-catenin-mediated binary decisions coupled with the first two animal–vegetal-oriented cell divisions diversify cell fates in the early *Ciona* embryo.

An ON–ON sequence specifies endoderm, an ON–OFF sequence induces margin, and an OFF–OFF sequence specifies ectoderm (Figure 5(b)).

Thus, reiterative β-catenin switches are used to diversify cell fates in very distantly related animals:

C. elegans (an ecdysozoan), *Platynereis* (a lophotrochozoan), and *Ciona* (a chordate). This suggests that this cell fate specification mechanism may have an ancient evolutionary origin.[4] Could a similar mechanism also play a role during vertebrate development? The Wnt/β-catenin pathway regulates the behavior of many types of stem cells in vertebrates. *In vivo*, β-catenin often promotes the maintenance of a stem cell identity while repressing differentiation.[31,32] In addition, *in vitro*, it has been observed that, when placed in contact with a bead covered by Wnt proteins, mouse embryonic stem cells orient their division relative to the Wnt source and divide asymmetrically.[33] The daughter cell close to the Wnt source accumulates β-catenin in the nucleus and remains a stem cell, while the daughter cell away from the Wnt source does not accumulate β-catenin and acquires a differentiated fate. During development of the cortex in the mouse embryo, neural stem cells (radial glial cells) undergo a succession of asymmetric divisions generating one daughter that differentiates into a neuron and one daughter that retains a stem cell fate.[34] β-Catenin transcriptional activity is high in neural stem cells, but low in newly generated neurons, and β-catenin promotes the maintenance of a stem cell identity while repressing neuronal differentiation.[35–38] The Wnt pathway also regulates the spindle-size asymmetry during cortical neural stem cells divisions.[39] These results raise the possibility that reiterative β-catenin switches play a role in the regulation of vertebrate stem cell lineages in the nervous system and potentially other tissues. However, further experiments, especially precise *in vivo* lineage tracing, are required to confirm this hypothesis.

GENERATION OF β-CATENIN ASYMMETRIES

How are these reiterative asymmetries of β-catenin generated? In *C. elegans*, the mechanism regulating β-catenin asymmetries has been studied more extensively during the asymmetric division of two cells: the EMS blastomere in the embryo (precursor of the endomesoderm) and the T cell in the larva (a precursor for epidermal and neural cells). Here I summarize the main concepts that have emerged from these studies. I refer the reader to recent reviews on Wnt signaling in *C. elegans* for further details on the Wnt/β-catenin asymmetry pathway.[7,40–42] The EMS and T cells are polarized before their division by Wnt ligands secreted by a posterior source

(Figure 6).[8,43–45] This leads to the asymmetric localization of Wnt pathway components to the posterior or anterior cortex of the cell. Wnt receptors (Frizzled) and Dishevelled proteins tend to be enriched at the posterior pole of the cell, while the APC protein APR-1, the Axin protein PRY-1, the Nemo-like kinase LIT-1, and the β-catenin WRM-1 tend to be enriched cortically at the anterior pole.[13,14,45–47] How Wnt ligands generate these asymmetries is currently unknown. These cortical asymmetries in the mother cell promote nuclear asymmetries of β-catenins between daughter cells. Interestingly, the nuclear asymmetries of the two β-catenins WRM-1 and SYS-1 are generated by different mechanisms. WRM-1 nuclear asymmetry is generated by a differential nuclear export between the two daughter cells.[13,14,47] APR-1/APC at the anterior cortex generates asymmetries in the microtubule cytoskeleton leading to the export of WRM-1 from the anterior nucleus and accumulation of WRM-1 in the posterior nucleus.[48] SYS-1 nuclear asymmetry is generated by its differential degradation. SYS-1 is degraded in the anterior daughter by the proteasome and this regulation involves the destruction complex components APR-1/APC and KIN-19/CK1.[15,16,22] In the posterior daughter SYS-1 is not degraded, enters the nucleus, and binds to the N-terminal domain of TCF transcription factor POP-1 converting it to an activator.[9,15,16] The fraction of POP-1 free of SYS-1 is recognized by WRM-1, which binds to the C-terminal domain of POP-1 leading to the phosphorylation of POP-1 by the Nemo Like Kinase LIT-1 and to export of POP-1 from the posterior nucleus.[12,49,50] Given that POP-1 can bind either SYS-1 or WRM-1, but not both of them at the same time, this mechanism ensures that all the POP-1 molecules in the posterior nucleus are associated with SYS-1 (any free POP-1 being exported) and generates an asymmetry in POP-1 concentration between the anterior and posterior nuclei.[50]

It is unclear at present whether the mechanisms that generate SYS-1 and WRM-1 asymmetries during the EMS and T cell divisions are used to generate all the reiterative SYS-1 and WRM-1 asymmetries observed during embryonic and larval development. Wnt ligands appear to play an important role in the regulation of many of these asymmetries. For example, several Wnt ligands coordinate the asymmetric divisions of the stem cell-like seam cells[51,52] or the P blasts cells[53,54] during larval development, and a Wnt ligand (MOM-2) regulates the orientation of many asymmetric divisions in the embryo.[55] However, how Wnt ligands can regulate the global coordination of asymmetric divisions in complex fields of
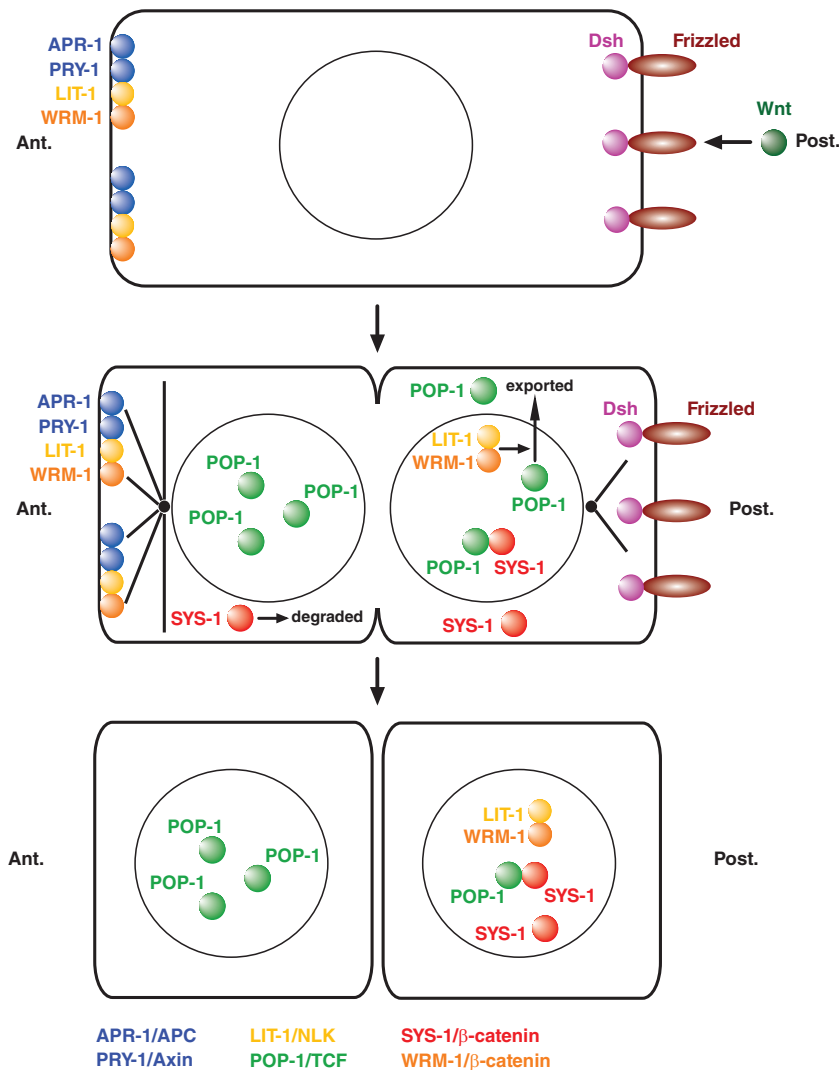
FIGURE 6 | Generation of β-catenin and TCF asymmetries in *C. elegans.* Model for the generation of the asymmetries of the β-catenins SYS-1 and WRM-1, and of the TCF factor POP-1 during asymmetric divisions. This model mostly derives from studies of the division of the EMS cell in the embryo and of the T cell in the larva.

cells is unknown and is an intriguing question. In addition, asymmetric localization of Wnt pathway components in the mother cell before division appears to be involved in many asymmetric divisions in the embryo and larva. For example, the Wnt receptor MOM-5/Frizzled is enriched at the posterior pole of many cells in the embryo.[56] In addition, several Wnt pathway components are enriched at the anterior or posterior pole of seam cells during larval development.[14,47] To conclude, both localized extracellular Wnt ligands and polarized intracellular cortical components appear to regulate the generation of β-catenin asymmetries in *C. elegans*.

How reiterative β-catenin asymmetries are generated during the development of *Platynereis* and *Ciona* is unknown. In both species, blocking the activity of the degradation complex using chemical inhibitors leads to an accumulation of β-catenin in both daughter cells and therefore to a loss of β-catenin asymmetry.[24,26] This suggests that the regulation of β-catenin by the destruction complex plays a key role in the generation of the asymmetry, similar to what is observed for SYS-1/β-catenin in *C. elegans*. Whether localized extracellular Wnt ligands and polarized intracellular cortical components also play a role is currently unknown. A recent analysis of the expression patterns of the Wnt ligands in the embryo of *Platynereis* revealed that none of them seem expressed in the right place at the right time to act as a global regulator of β-catenin asymmetries, suggesting a possible Wnt ligand independent mechanism in this organism.[57]

INTEGRATION OF β-CATENIN ASYMMETRIES INTO GENE REGULATORY NETWORKS

How reiterative β-catenin asymmetries are converted into diverse cell fates has been studied in both *C. elegans* and *Ciona* but not in *Platynereis*. In the *C. elegans* embryo, different sets of lineage specific transcription factors are expressed in the anterior versus posterior daughter cells at each successive division.[58] Each cell expresses a unique set of transcription factors different from the one of its mother cell or its sister cell, and the generation of these diverse regulatory states is regulated by the Wnt/β-catenin asymmetry pathway (Figure 7(a)).[17,58] How the Wnt/β-catenin asymmetry pathway can regulate the expression of such a diverse set of transcription factors has been analyzed in several specific lineages of the embryo and the larva. Analysis of the *cis*-regulatory regions of genes activated in posterior daughters has revealed that they contain binding sites for the TCF transcription factor POP-1. This is the case for the GATA transcription factor gene *end-1* (embryonic endomesoderm lineage EMS),[59,60] the homeodomain transcription factor gene *ceh-10* (embryonic neuronal lineage AIY),[18] the Meis transcription factor gene *psa-3* (larval T blast cell

lineage),[61] the Nkx transcription factor gene *ceh-22* (larval distal tip cell precursor lineage),[62] or the GATA transcription factor gene *egl-18* (larval seam cell lineage).[63] They are directly activated by the POP-1:SYS-1 complex in the posterior daughter and repressed by POP-1 in the anterior daughter. These transcriptional targets are specific to a given lineage and the POP-1:SYS-1 complex cooperates with lineage specific transcription factors inherited from the mother cell to determine which specific targets will be activated in a given posterior daughter cell. For example, in the EMS lineage, the POP-1:SYS-1 complex cooperates with the bZip transcription factor SKN-1 and the GATA transcription factor MED-1, inherited from the mother cell, to activate *end-1* expression.[59] In the T lineage, POP-1:SYS-1 cooperates with the Hox transcription factor NOB-1 and the Pbx transcription factor CEH-20, received from the mother cell, to activate *psa-3*.[61] Finally, in the AIY neuronal lineage, POP-1:SYS-1 cooperates with the homeodomain transcription factor TTX-3, inherited from the mother cell, to activate *ceh-10*.[18] The general asymmetric division cue (POP-1:SYS-1) and the lineage-specific determinants (lineage-specific transcription factors inherited from the mother cell) are directly integrated at the level of the *cis*-regulatory regions of the target genes *end-1*, *psa-3*,
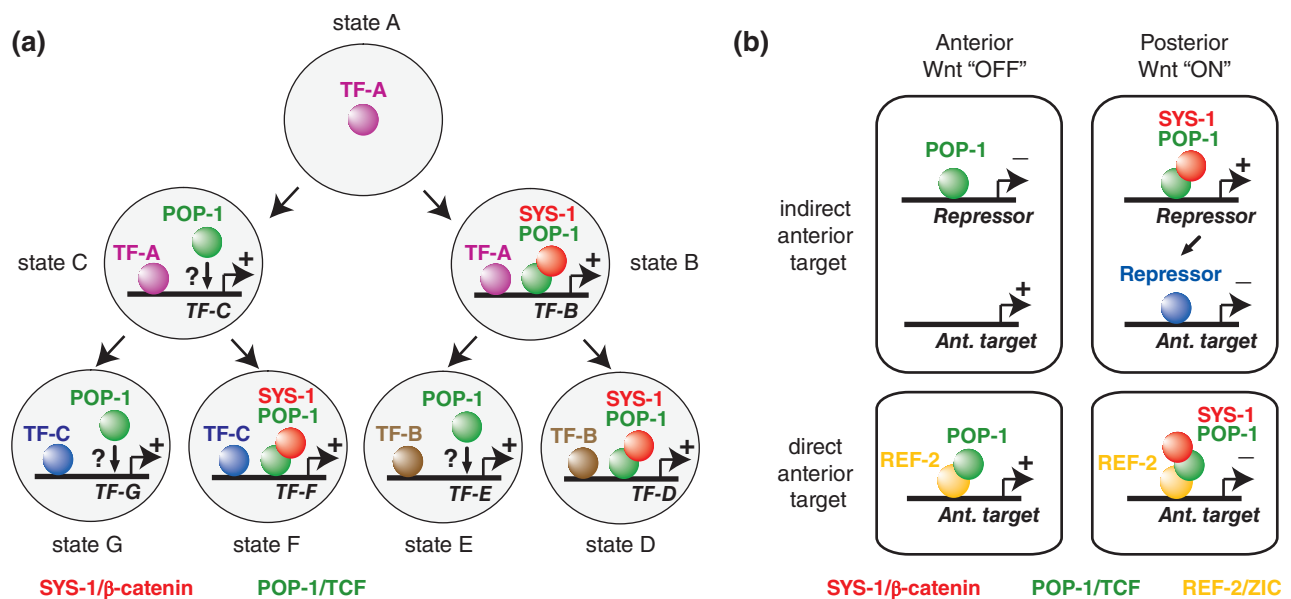

FIGURE 7 | Model for the integration of β-catenin asymmetries into the gene regulatory network of *C. elegans*. (a) Model for the generation of novel regulatory states during two rounds of division. For details on the mechanism of transcriptional activation of target genes in anterior daughter cells by POP-1 (?) see text and panel (b). (b) Transcriptional activation of target genes in Wnt 'OFF' (anterior) cells. This regulation can be either indirect (via the repression of a posterior repressor) or direct (via the formation of a POP-1:REF-2 protein complex).

and *ceh-10*, which contain a combination of binding sites for these factors (Figure 7(a)).[18,59,61]

While the mechanism of transcriptional activation of posterior target genes in posterior daughters is well studied, the mechanism whereby anterior target genes are specifically activated in anterior daughters is less well understood.[64] One simple mechanism could be that the POP-1:SYS-1 complex directly activates the expression of a transcriptional repressor in the posterior daughter; this repressor then represses the expression of anterior targets in the posterior daughter, therefore restricting their expression to the anterior daughter (Figure 7(b)). There is indeed evidence for such a mechanism in some lineages. For example, in the EMS lineage, the expression of the transcription factor END-1 is directly activated in the posterior daughter by the POP-1:SYS-1 complex, and END-1 represses the expression of some anterior daughter cell markers in the posterior daughter.[65,66] Similarly, in the terminal division of the AIY neuronal lineage, the expression of the transcription factor CEH-10 is directly activated in the posterior daughter by the POP-1:SYS-1 complex, and CEH-10 represses the expression of some anterior daughter cell markers in the posterior daughter.[18] However, how END-1 or CEH-10 represses anterior daughter markers is unknown. Recently, a more direct mechanism of regulation for anterior target genes has been described.[19] In an anterior daughter (the SMDD/AIY neuroblast), expression of the *ttx-3* gene is activated via a binding site for a Zic transcription factor, REF-2 (Figure 7(b)). In the anterior daughter, the POP-1 protein associates with the REF-2 protein and the POP-1:REF-2 complex activates *ttx-3* expression via the Zic binding site in the *ttx-3 cis*-regulatory region. In the posterior daughter, SYS-1 binding to POP-1 blocks this activation. Therefore, POP-1 can directly regulate the transcription of an anterior target gene without an intermediate step of transcription. This more direct mode of regulation may be an advantage in the fast developing *C. elegans* embryo, where time between successive cell divisions is very short (around 30 min),[5] as it avoids the time delay of an additional transcriptional intermediate step (transcriptional activation/repression of the repressor gene). Whether a similar regulatory logic applies to other anterior targets remains to be determined.

Details of how reiterative β-catenin asymmetries are integrated into the gene regulatory networks that diversify cell fate are also beginning to emerge in *Ciona* (Figure 8). Following the first β-catenin asymmetry, which segregates the ectoderm (animal daughters) from the mesendoderm (vegetal daughters), β-catenin activates the expression of target genes,

such as the Fox transcription factors gene *FoxA* and *FoxD*, in the vegetal daughters,[29,67] and restricts the expression of ectodermal genes, such as the *Fog* transcriptional coactivator gene, to the animal daughters by repressing them in the vegetal region.[30] During the second β-catenin asymmetry, which segregates the margin (animal daughters) from the endoderm (vegetal daughters), both β-catenin and FoxA are required to activate the expression of endodermal transcription factors, such as *Lhx3*, in the vegetal daughter.[26,68,69] In the animal daughters, where β-catenin is absent, FoxA and FoxD are required to activate the expression of marginal transcription factors, such as *ZicL*.[26,69–71] Therefore, as in the case of *C. elegans*, reiterative β-catenin asymmetries diversify cell fates by establishing novel transcriptional states in each daughter cell after each successive division. These novel transcriptional states result from the integration of the β-catenin switch with lineage specific transcription factors inherited from the previous division step. Similar to what has been observed in *C. elegans*, β-catenin directly activates target genes in the daughter cells where it accumulates, via TCF binding sites present in their *cis*-regulatory regions, as shown in the case of the target gene *FoxD*.[29] How target genes are activated in daughter cells where β-catenin is absent is less clear. Interestingly, genes activated in the early ectoderm, where β-catenin is absent (such as *Fog*) contain binding sites for a maternal GATA transcription factor, GATAa.[30,72] While the GATAa protein is present ubiquitously, its ability to activate transcription is blocked in the vegetal region by β-catenin and TCF.[30] It will be interesting to determine whether the β-catenin:TCF protein complex directly represses the activity of GATAa by binding to the GATAa protein, following a regulatory logic very similar to the regulation of anterior target genes by the Zic transcription factor REF-2 in *C. elegans*.

CONCLUSION

Recursive β-catenin switches are used to diversify cell fate in very distantly related animals (*C. elegans*, *Platynereis*, and *Ciona*), suggesting that this mechanism may have an ancient evolutionary origin. The Wnt pathway, by its ability to regulate both the orientation of cell divisions and the fate of the daughter cells produced, may be particularly well suited to regulate the development of systems where the lineage is fixed and the position of cells is stereotyped such as the early embryos of *C. elegans*, *Platynereis*, and *Ciona*. It will be interesting to determine whether reiterative
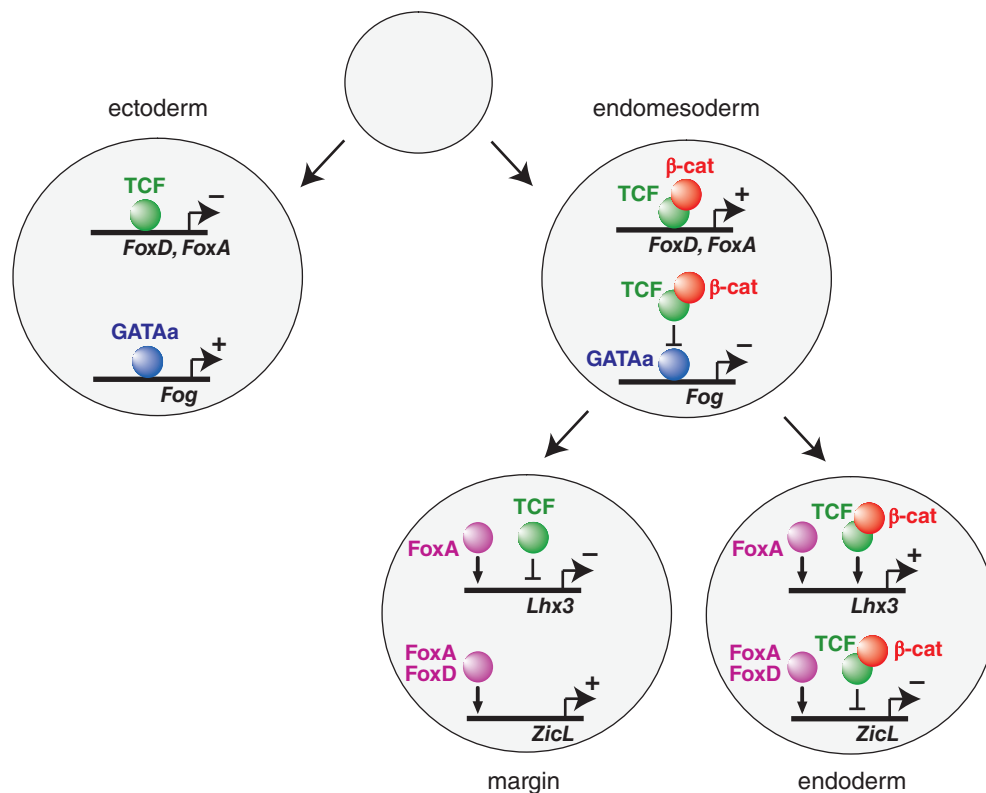
FIGURE 8 | Model for the integration of β-catenin asymmetries into the gene regulatory network of *Ciona*. The regulations of the *FoxD* gene by TCF:β-catenin and of the *Fog* gene by GATAa are direct. Whether the other regulations are direct remains to be determined.

β-catenin switches are also used to diversify cell fates in other animals. In particular, in vertebrates, several stem cell lineages (such as neural stem cells) seem a promising place to look. The progress of live imaging methods *in vivo* and the development of organoid methods *ex vivo* should help to determine whether reiterative β-catenin binary decisions are used during the generation of organs by vertebrate stem cells.

How these global patterns of reiterative β-catenin asymmetries are generated in time and space is an intriguing question. Studies in *C. elegans* suggest that the localized expression of Wnt ligands can play a key role in the regulation of these patterns. However, the molecular mechanism by which Wnt ligands can coordinate these global patterns of β-catenin asymmetries across the embryo is unknown and is an important area of investigation in the future. Studies in this area will also bring important information into the broader question of tissue polarization by Wnt signaling, a process present in many animals.

Following each division, the asymmetry in β-catenin levels is used to activate different sets of target genes in each daughter cell. This implies that β-catenin and TCF have a very large number of target genes, each of them being activated in different lineages and at different time points. How TCF and β-catenin reach such a specificity of target activation is an intriguing question. Part of this specificity is due to the presence, in the *cis*-regulatory regions of the target genes, of binding sites for lineage specific transcription factors in close proximity to TCF binding sites, both type of sites contributing to the transcriptional activation. In addition, TCF can also make direct protein–protein interactions with lineage specific factors (such as the Zic factor REF-2), which can modulate its activity in a lineage and temporal specific manner. In the future, it will be important to determine the full set of lineage specific factors with which TCF and β-catenin can interact. Given the key roles played by TCF and β-catenin in multiple pathologies, such as cancer, identifying novel interactors or new mode of actions for these essential factors will certainly have important implications for the understanding of human diseases.

ACKNOWLEDGMENTS

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