Molluscan Models: *Crepidula fornicata*

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Abstract:

Gastropod snails in the genus *Crepidula* have emerged as model systems for studying a metazoan super clade, the Spiralia. Recent work on one species in particular, *C. fornicata*, has produced high-resolution cell lineage fate maps, details of morphogenetic events during gastrulation, key insights into the molecular underpinnings of early development, and the first demonstration of CRISPR/Cas9 genome editing in the Spiralia. Furthermore, invasive species of *Crepidula* are a significant ecological threat, while one of these, *C. fornicata*, is also being harvested for food. This review highlights progress towards developing these animals as models for evolutionary, developmental, and ecological studies. Such studies have contributed greatly to our understanding of biology in a major clade of bilaterians. This information may also help us to control and cultivate these snails.

Crepidula, model representatives of the Spiralia

The genus *Crepidula* comprises the “slipper snails”, members of the caenogastropod family Calyptraeidae within the Gastropoda (**Fig 1A-B**). Adult slipper snails have a limpet-like morphology with a posterior apex and internal septum that covers much of the visceral mass (**Fig 1A-B**; Collin and Cipriani, 2003; Henry et al., 2010a). The presence of this internal shelf gives the shell the appearance of a small slipper when the animal is removed. As members of the Mollusca, slipper snails represent one of 14 phyla that comprise the metazoan superphylum Spiralia, the largest clade of bilaterian metazoans (**Fig. 2A**). Molluscs represent the second most speciose animal phylum and slipper snails lie within the molluscan class Gastropoda (**Fig. 2B**). Spiralia exhibit diverse larval and adult body plans, yet many representatives share a stereotypical mode of development including a conserved fate map and cleavage pattern, referred to as spiral cleavage (**Figs. 2, 3A-E**; Henry, 2014). In fact, Edwin G. Conklin (1897) formalized the spiral cleavage cell lineage nomenclature while studying *Crepidula* at the Marine Biological Laboratory in Woods Hole, MA. The conserved spiral cleavage pattern offers a unique opportunity to discover how diverse body plans evolved from a similar starting point, by studying homologous cell lineages at the single cell level. Yet, compared to deuterostomes and ecdysozoans, spiralian have received much less attention as subjects for understanding cell and molecular mechanisms controlling development.

Models for studying life history strategies

The *Crepidula* genus contains nearly 60 species, and their phylogenetic relationships have been well established (Hoagland, 1977, 1984, 1985; Collin, 2000a, 2001, 2003a, b, 2004; Henry et al., 2010). These species exhibit a wide array of life history strategies. For instance, species such as *C. fornicata* exhibit indirect, planktotrophic development (**Fig 1A, C**), while others, including *C. convexa* and *C. atrasolea*, have life cycles that involve direct forms of development (**Fig. 1B, D-G**, Collin, 2000b, 2001, 2003c, 2004). Species like *C. onyx* and *C. navicella* exhibit adelphophagic modes of development where embryos consume sibling, non-developing or aborted nurse eggs and embryos contained in the egg capsules (Collin and Spangler, 2012;

Lesoway et al., 2014). Adelphophagy could permit retention of larval features and provide flexibility for reinvention of planktotrophic modes of development, which is seen in some other closely related species (Collin, 2004; Lesoway et al., 2014). The wide range of developmental strategies and solid phylogenetic framework make this group well-suited for studies to examine life history evolution. *Crepidula* have also been used to examine larval development, growth and metamorphosis, with many studies examining how metamorphic competence arises in planktotrophic forms (e.g., Pechenik et al., 1996a,b, 2007; Taris et al., 2009). The larva must grow to a certain size to reach metamorphic competence, and a recent study by Padilla et al. (2014) using *C. fornicata* found that achieving metamorphic competence is related to food abundance, regardless of diet composition or temperature.

Models for studying sex change

Calyptraeid snails have also been used to study sexual development and sex change (**Fig 1A-B, G**). They are protandric hermaphrodites, which means that following an initial juvenile phase, all individuals first become reproductive males and subsequently transition to reproductive females. Isolated males will transition to females more quickly, and females grow at a much faster rate. The presence of females delays males from becoming females (Coe, 1936, 1938, 1953; Hoagland, 1978; Collin et al., 2005). Recent studies found that the factor(s) controlling sex change and growth in *C. fornicata* appear to be contact-dependent, and not water-borne substances, as had been previously assumed (Cahill et al., 2015; Carrillo-Baltodana and Collin, 2015).

Invasive *Crepidula*—pest or pastry?

C. fornicata is native to the American north eastern Atlantic, but has been introduced into the waters of Europe, including the Mediterranean Sea, and the Northern Coast of France, Spain, the North Sea, the British Isles, Japan, Uruguay, and the West and Pacific Northwest coastal areas of America (Orton, 1912a; Spärck, 1935, 1950; Korringa, 1942; Chippenfield, 1951; Walne, 1956; Hessland, 1951; Louis, 1963; Dommasnes and Schram, 1973; Barnes et al., 1973; Minchin et al., 1995; Sjøtun, 1997; Blanchard, 2009; Chauvaud et al., 2000; Hedeselskabet, 2003; Thieltges et al., 2003; Nehls, et al., 2006; Collin et al., 2006; 2009; Riera, 2007; Viard et al., 2006; Moulin et al., 2007). Reports indicate that *C. fornicata* may have first appeared in European waters sometime towards the end of the 19th century, presumably associated with the importation of American Shellfish (e.g., oysters). Specimens may have also been attached to the hulls, or released from ballast water of Allied warships visiting the region during the World Wars (Walne, 1956; Minchin et al., 1995; Blanchard, 1997; Sauriau et al., 1998; Richard et al., 2006; Minchin, 2007; Rayment, 2008). Another species, *C. onyx*, which is native to Southern California, has invaded waters of the Pacific Northwest, and Hong Kong (Huang et al., 1983; Holm, 2006).

There is tremendous concern over the ecological impact of these invasive snails (Viard, 2010). *Crepidula* are highly effective filter feeders that compete for available phytoplankton (Johnson, 1972; Newell and Kofoed, 1977; Beninger et al., 2007; Decottignies et al., 2007a; Blanchard et al., 2008). Their feces and pseudofeces (undigested food) also alter turbidity and surrounding sediment beds (Loomis & van Nieuwenhuyze 1985; Ehrhold et al., 1998; le Pape et al., 2004), and the adults are highly fecund, with juveniles settling on hard substrates, including local shellfish to compete for space and thus, act in a bio-fouling capacity (de Montaudouin et al., 1999; Thieltges 2005b; Thieltges and Buschbaum, 2007; Decottignies et al., 2007a,b; Frésard and Boncoeur, 2006; Blanchard, 2009). These factors have contributed to large changes in foreign ecosystems, with significant impacts on local shell fisheries and fish populations (de Montaudouin et al., 1999; de Montaudouin and Sauriau, 1999; Thouzeau et al., 2000; Valett et al., 2001; Grall and Hall-Spencer, 2003; Thieltges, 2005a, Thieltges et al., 2006; Frésard and

Boncoeur, 2006; Martin et al., 2007; Moulin et al., 2007; Reise and van Beusekom, 2008; Molnar, et al., 2008).

On the other hand, the slipper snail is now showing up in restaurants in France, Japan and Spain, having been harvested from those areas in which they have invaded (Clozel, 2014). Diners there are not the first to try these shellfish, as slipper snails have been consumed by people of the Azores and by members of the Portuguese community that settled in America along the Atlantic coast, including in New England, where they refer to them as “sweet meats” or “Lappas” after the limpets consumed in Portugal. These snails have a high nutritional content (Dagorn et al., 2014) and are flavorful (Mahaney et al., 2007). In France, *Crepidula* is touted as a culinary delicacy, where they are called “Berlingot des mers”, for “pastries” or “candies of the sea” (see **Fig 1H**; Clozel, 2014). A better understanding of the molecular underpinning of embryonic and larval biology would be useful both for controlling and raising these snails.

Insights into the cellular and molecular basis of *Crepidula* development

The embryos of *Crepidula* are well-suited for studies on developmental biology, particularly at the cell and molecular levels (Henry et al., 2006, 2010a-d; Henry and Perry, 2008). The cell lineage has been well characterized (Hejnal et al., 1997; Lyons et al., 2012, 2015). Precise cell lineage ablation can be accomplished either by hand or using a laser (Lyons et al., 2012). One can readily perform cell/molecular functional studies, as embryonic cells are easy to inject (Henry et al., 2010d; Lyons et al., 2015), and one can thus introduce synthetic RNAs encoding various proteins and dominant negatives, or morpholino oligonucleotides, for gain- and loss-of-function studies (Henry et al., 2010d; Lyons et al., 2015). At least four EST data sets are available, covering a wide range of developmental stages to identify genes of interest (Henry et al., 2010c; Perry et al., 2015). Such studies have revealed a great deal about fundamental mechanisms that regulate development.

For example, classically, embryos with stereotyped cell lineages, such as spiralian, were assumed to specify cell fates by reliance on asymmetrically localized, cell-autonomous determinants. In *C. fornicata*, like its close relative *Ilyanassa obsoleta* (Lambert and Nagy, 2002), certain mRNAs are asymmetrically localized to specific cells, by virtue of their transient association with the centrosome and cell cortex (Henry et al., 2010c). However, detailed experiments using direct cell lineage tracing, experimental embryology, and investigations of the molecular underpinnings of development, have revealed that spiralian also employ inductive signals to specify cell fates and axial properties (Henry, 2014). Experiments have shown that the dorso-ventral axis is specified by inductive interactions from a key organizer in spiralian that arises from the D (dorsal) cell quadrant; most typically it is the 3D macromere and/or its daughter the 4d micromere that serves as the organizer (**Fig. 3D-I**, Henry, 2014). By removing specific cells one can assess the precise cellular and chronological basis of D quadrant specification, as well as subsequent organizer signaling. In equal-cleaving spiralian, such as *Crepidula*, any one of the third quartet macromeres can be specified to become 3D, which occurs soon after the birth of the third quartet of micromeres (3a-3d) at the 20-cell stage (Henry et al., 2006; see **Fig. 3D,G**). 3D specification involves contacts with progeny of the first quartet (1q) micromeres (**Fig 3F-G**). Subsequently, the 3D macromere divides to form its daughter, 4d cell, at the 25-cell stage (**Fig 3E, H**). Once born, 4d serves to quickly signal the adjacent micromeres (**Fig 3I**). In *C. fornicata*, removal of the tiny polar lobe, a cytoplasmic protrusion inherited by the future D blastomere at the four-cell stage, leads to embryos with supernumerary D quadrant cells (i.e., “twins” or “triplets”, Henry and Perry, 2008). Therefore, contents of the polar lobe are not essential to forming the D quadrant, but instead bias selection of only one D quadrant (**Fig 3F-G**). This may involve lateral inhibition to the three adjacent quadrants, though the molecular nature of those signals is not understood (**Fig 3G**). These data support a more

complicated, multistep model of D quadrant specification and organizer activity, which involves both asymmetrically localized factors, and induction, than had been previously understood (Henry, 2014, **Fig. 3F-I**).

Studies are beginning to reveal the signaling cascades that regulate cell fate specification in *Crepidula*. MAPK signaling has been shown to be responsible for the specification of the D quadrant macromere 3D in both *Crepidula* and *Ilyanassa* (Lambert and Nagy, 2001, 2003; Henry and Perry, 2008), but not for 3D or 4d organizer activity *per se*. Other investigations reveal that gastrulation as well as the ultimate differentiation of endomesodermal fates derived from 4d in *Crepidula*, rely on active β -catenin signaling (Henry, et al., 2010d, unpublished data), which is consistent with the widely conserved role of β -catenin signaling in endomesoderm specification (Henry et al., 2008, 2010a).

What have *Crepidula* cell lineage studies taught us?

Modern cell lineage studies have used direct labeling of individual cells with fluorescent lineage tracers to construct detailed fate maps in slipper snails (Hejnal et al. 2007; Lyons et al. 2012; Lyons et al. 2015). For example, a recent study of the sublineages of the 4d cell, which was known to be the progenitor of the bi-potential endomesoderm germ layer, revealed the exact origin of distinct tissues including hindgut, heart, muscles, larval kidney complex, and the primordial germ cells (Lyons et al., 2012). Characteristic of many spiralian, after its formation, 4d divides to form the paired left and right teloblasts that generate both endoderm and mesoderm (endomesoderm). Fate mapping studies revealed that specific endodermal and mesodermal fates are segregated over a much greater number of teloblast divisions than had been previously known. Ablation experiments of those daughter cells showed that cell fates are established at birth, and no other cells can regulate to replace the missing tissues. These data suggest that the teloblasts have some mechanism to “count” cell divisions and perhaps segregate specific determinants to daughter cells with particular birth orders. A similar phenomenon has been demonstrated in the teloblasts of the annelid leech (Martindale and Shankland, 1990), but the underlying molecular mechanisms in both groups remain unknown.

Lineage tracing studies were also able to answer long-standing questions about gastrulation in the spiralian, including which lineages give rise to the mouth and anus, and the cell behaviors involved (Hejnal and Martindale, 2009, Lyons and Henry 2014). In *Crepidula*, gastrulation proceeds by epiboly, and the cells that contribute to the lip of the blastopore have been followed, including the progeny of the 2nd and 3rd quartet of micromeres (**Fig 4A-E**, Lyons et al., 2015). This study redefined the blastopore lip as the boundary between ectoderm and mesoderm/endomesoderm (Lyons et al., 2015), and showed that *C. fornicata* is a *bona fide* protostome (i.e., the cavity that leads to the embryonic gut or archenteron becomes the opening to the mouth, see also Chan and Lambert, 2014). This study also firmly established that the anus (derived from the 2d lineage) forms at a site separate from the blastopore, which challenges certain evolutionary hypotheses, including the amphistomy hypothesis (Arendt and Nübler-Jung, 1997; Arendt, 2004; Lyons et al., 2015). The amphistomy hypothesis posits that protostomes and deuterostomes arose from an amphistomous ancestor in which both the mouth and the anus arose from the blastopore. Though some might interpret the events taking place in *Crepidula* to support the amphistomy hypothesis (as described by Arendt and Nübler-Jung, 1997; Arendt, 2004), we argue there is a lack of direct evidence to support that proposed series of events (Lyons et al., 2015). Unlike the mouth, the anus does not form from a persistent opening of the posterior blastopore in *Crepidula*. Although the anus is derived from progeny of 2d, only a few derivatives of this expansive clone initially lie along the posterior edge of the animal cap of ectoderm (e.g., 2d122 and 2d22, see Conklin, 1897) and those cells are displaced from the blastopore lip prior to the formation of the archenteron (Lyons et al., 2015). The

opening of the anus actually forms several days later, presumably under the influence of inductive interactions with the terminal hindgut endoderm (Lyons et al., 2012, 2014, 2015).

Studies of developmental dynamics

C. fornicata embryos develop slowly and express fluorescently tagged proteins from injected mRNA, making them excellent material for live imaging (**Fig 4C-E, K-O**, Lyons et al., 2015). The combination of global expression of nuclear and cytoskeletal biosensors with fluorescent lineage tracers in specific cell lineages revealed the unique gastrulation behaviors of different cells. The 4d-derived endomesodermal clone is internalized passively, initially by the expansion of the ectodermal cap (**Fig 4K**), and later by the closure of the posterior lip of the blastopore. This latter process occurs by a posterior-to-anterior zippering of the 3d² and 3c² sublineages starting at the posterior lip of the blastopore (**Fig 4F-J**), and represents a form of convergent-extension. This morphogenetic process is likely modified in various spiralian groups, giving rise to the great variation of gastrulation modes in this clade (Arendt, 2004; Hejnal and Martindale, 2009, Lyons and Henry 2014). A different form of convergent-extension involving another cell lineage, the ventral trunk neuroectoderm, has been described from later stages of development in the annelid *Platyneries* (Steinmetz et al., 2007).

In contrast to the 3d² and 3c² sublineages in *C. fornicata*, cells along the anterior edge of the blastopore lip (3a² and 3b²) give rise to a second population of mesoderm derived from ectodermal precursors (called “ectomesoderm”) (Lyons et al. 2015). These ectomesodermal progeny undergo an epithelial-mesenchymal transition (EMT), and extend filopodia and lamellipodia as they pass deep inside the embryo to migrate away from their site of internalization (**Fig 4M-O**). Imaging studies also suggest that the paired 2m^{2.2} sublineages of endomesodermal 4d cell gives rise to the germline. By labeling the actin cytoskeleton one can observe the primordial germ cells’ dynamic behaviors to form long filopodia and lamellipodia that are presumably associated with directed migration towards the endoderm and future gonads (Lyons et al., 2012).

Future directions

A reliable and robust method for perturbing gene function during embryonic, larval, and adult stages is required to gain a mechanistic understanding of *Crepidula* biology. Both morpholino oligonucleotides, and dominant negative constructs, have been used successfully in the past (Henry et al., 2010 a,b; unpublished observations), but these methods have limited effectiveness at later stages. This impediment can be overcome through genome editing using CRISPR/Cas9 technology, which has recently been demonstrated in *C. fornicata*, where mCherry was coupled to the endogenous β -catenin gene (**Fig. 5A-C**, Perry and Henry, 2015). This permitted long-term observation of β -catenin expression, and represents the first demonstration of CRISPR/Cas9-mediated transgenesis in any member of the superphylum Spiralia. CRISPR/Cas9 technology provides a tool for both knocking genes out to assess gene function in the F0 generation, and for making stable transgenic lines of mutant snails or knock-in snail lines carrying various reporters. The only remaining hurdle is the lack of a sequenced genome. With preliminary estimates placing the *C. fornicata* genome at around 2.6pg (Paola Pierossi, Gregory Lab, Univ. of Guelph, personal communication), it should not be long before it is sequenced.

Perturbing gene function will permit the construction of gene regulatory networks (GRNs) to understand cell fate specification and morphogenesis. For example, one open question is the evolutionary relatedness of spiralian ectomesoderm and endomesoderm (Boyer et al., 1996; Lyons and Henry, 2014; Perry et al., 2015). Endomesoderm is argued to be an ancient bi-potential tissue type shared by metazoans, and a conserved gene regulatory network (GRN)

controls specification of endomesodermal territories in deuterostomes (Davidson et al., 2002a,b; Oliveri and Davidson, 2004; Smith and Davidson, 2008; Croce and McClay, 2010; Croce et al., 2011; Peter and Davidson, 2011; Solnica-Krezel and Sepich, 2012), ecdysozoans (Maduro, 2006; Gorfinkel et al., 2011) and cnidarians (Röttinger et al., 2012). The ectomesoderm cell population may represent a novel innovation in the Spiralia (Boyer et al., 1996; Lyons and Henry, 2014; Chan and Lambert, 2014; Henry, 2014). A recent comparison of transcription factors expressed in *C. fornicata* ectomesoderm versus endomesoderm revealed that while many regulatory factors are expressed in both tissues, others are uniquely expressed in one or the other. For example, the genes *snail2* and *twist* are expressed only in ectomesoderm, which may reflect their EMT behavior during gastrulation (Perry et al., 2015). Systems-level GRN analysis can uncover what upstream regulatory inputs regulate differential expression between ectomesoderm and endomesoderm, and whether removing particular genes like *snail2* and *twist* block EMT. These types of data are necessary for making evolutionary inferences about the origins of novel cell types like the ectomesoderm.

The origins of the somatic gonad and the molecular underpinning of sex determination are wide open questions as well. For these studies, a direct developing species, such as *C. atrasolea*, will be useful, as their egg-to-egg generation time (6 months) is significantly faster than that in *C. fornicata* (well over a year, Henry and Lyons, unpublished observations; Rachel Collin, personal communications). *Crepidula* species, such as *C. atrasolea*, are easy to rear through successive generations in closed aquaria (**Fig 1B, D-G**, Henry et al., 2010; Pires, 2014; and our unpublished data). Another fruitful area for future research is comparisons of indirect versus direct development. The ability to compare species with different life history strategies, using a shared stereotyped cell lineage, will allow fine-grain comparisons of differences in morphology (e.g., Lyons et al., 2012). Modes of gastrulation (e.g., invagination vs. epiboly) are often tied to egg size (small vs. large eggs) and life history strategies (indirect-larval vs. direct development, Lyons and Henry, 2014). The wide range of developmental modes in the *Crepidula* genus offers future avenues for understanding how gastrulation and germ layer specification (e.g., ectomesoderm, which contributes largely to larval structures) may have changed over the course of these developmental transitions (Lyons and Henry, 2014). Ultimately, information gained from developmental studies might be employed in an effort to both rear these snails as a source of food as well as to control invasive populations of *Crepidula* in the wild.

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Figure Legends:

Figure 1. A. two curved stacks of adult *Crepidula fornicata*. Large individuals are females while the smaller ones are males, as labeled. B. Adult *C. atrasolea* that have settled on a clam shell. Unlike *C. fornicata*, these animals do not form stacks. C. Indirect developing, planktonic,

veliger larva of *C. fornicata*. Notice the large ciliated velum, which is eventually lost at metamorphosis. D. transparent egg sac containing four direct-developing (non-feeding) *C. atrasolea*. These have a greatly reduced velum, and hatch as crawl-away juvenile snails. Simple process for rearing *C. atrasolea* snails in the lab. C. juvenile snails (jv) being reared in small petri dishes. Once they reach sizes greater than 2mm they are transferred to floated chambers with screened end caps (F) where they continue to be fed commercial dead phytoplankton (Phytofeast). G. Close-up view of the culture chamber with one end cap removed. Notice the two larger gravid females (black arrowheads), surrounded by smaller males. H. Tasty salad appetizer of *Crepidula* sautéed in white wine, butter, garlic and saffron, served with a flavorful dressing, over a bed of mesclun and other vegetables, garnished with finger lime pulp. ft, foot; oc, ocelli; sh, shell; tn, tentacle; vl, velum. Scale bars equal 1cm for A and B, 50µm for C, and D, 1cm for F, and G.

Figure 2. A. Phylogenetic relationships between various Lophotrochozoa/Spiralia. These phylogenetic relationships are somewhat unclear, and this phylogram is based on a recent consensus reached by several authorities (see review by Henry, 2014). This view does not take into account the findings of Struck et al. (2014), which would remove the Gnathozoa” or “Gnathifera” from the platyzoan clade, and make additional changes to the topology of this tree. Other enigmatic groups with uncertain relationships include the Acanthocephala (thought to be related to the Rotifera as Syndermata), the Myzostomida (thought to represent derived annelids), and the Mesozoa. B. One proposed phylogeny for the Mollusca (from Kocot et al., 2011). Phylogenetic relationships amongst the Mollusca are also highly contentious (see Sigwart and Lindberg, 2014; and Williams et al., 2014; Osca et al., 2015 for relationships within the Gastropoda). The various named clades are labeled for particular nodes. Phyla and classes that contain species exhibiting spiral cleavage are underlined in green. Figure modified after that of Henry (2014).

Figure 3. A-E. Animal pole views of spiral cleavage, showing birth of the successive micromere quartets within the four basic cell quadrants (termed A-D by convention). Dorsal (D quadrant) is down, ventral (B quadrant) is up. Micromere daughters are labeled with lower case letters. For instance the first quartet micromeres (generically called 1q cells) are named 1a, 1b, 1c, and 1d. When those cells divide their animal-most daughters (collectively called the 1q¹ cells) are named 1a¹, 1b¹, 1c¹, and 1d¹. The vegetal-most large macromeres are labeled with upper case letters (e.g., 1A, 1B, 1C, 1D). Ultimate germ layer fates for these cells are indicated, as shown in the color key (illustrations after Lyons and Henry, 2014). Darker colors signify the initial birth of particular micromere quartets. Grey = mother cells (e.g., macromeres) prior to the generation of all four of their micromere daughters. F-I. Model showing steps involved in D quadrant specification and 4d organizer activity in *Crepidula* (illustrations after Henry, 2014). Signaling interactions (factors, lines and arrows) implicated in some events are noted with corresponding colors. The molecular basis of some signaling interactions are undetermined (?).

Figure 4. A-B. Micromere specific clones of the blastopore (bp). Clones labeled, as colored. F-J. Process of gastrulation (epiboly) and blastopore constriction involving convergent-extension of 3c² and 3d² progeny (enclosed in the shaded boxes shown in F and J of the larger red and green fluorescently labeled 3c and 3d clones, as labeled). Location of the constricting blastopore (bp) is indicated by dotted lines. The blastopore ultimately gives rise to the mouth (protostomy). Anterior is towards the top of the figure. K clone of 4d derived endomesoderm (red dil fluorescence) is seen underneath the advancing cap of ectoderm (green fluorescence). Animal pole is towards the top of the figure. L-O. Formation of ectomesoderm from 3b. Cells derived specifically from the 3b² subclone undergo EMT to form mesenchyme visualized by the wondering dil labeled progeny (red). For L-O the views are as marked in O (illustrations after

Lyons et al., 2015). Embryonic cells outlined with Utrophin-GFP (green fluorescence) as an F-actin binding biosensor, and nuclei labeled with Histone H2B-mCherry (red fluorescence) are seen in the embryos shown in C-E and K-O, as indicated. Scale bar in E equals 50µm.

Figure 5. Successful transgenic integration showing expression of *mCherry*-tagged β -catenin in *C. fornicata* embryos. (A) An embryo in the mid-stages of gastrulation showing mosaic expression of β -catenin-mCherry fluorescence in what appears to be a 4th quartet macromere (4Q) and its daughter (4q) macromere daughters of the D-quadrant (ventral view). Some nuclear localization is seen in two of these cells (arrowheads). (B) Brightfield and corresponding fluorescence (C) inferior view of a 14-day old veliger larva. Expression is observed in cells of the right and left mid-gut glands, and in two other cells lying adjacent to the hindgut intestine, that could be the two primordial germ cells. Nuclear localization is seen in many of the endodermal cells. bp, blastopore; ft, foot; int, intestine; lmg, left mid-gut gland; pgc, putative primordial germ cells; rmg, right mid-gut gland; sh, shell; vl, velar lobes. Scale bar in D represents 50µm for parts A and 60µm for B and C. Figure after Perry and Henry (2015).

figure 1

A

B

figure 2

figure 3

figure 4

figure 5