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Lucas Leclère, Richard R. Copley, Tsuyoshi Momose and Evelyn Houliston

Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire de Biologie du Développement de Villefranche-sur-mer (LBDV), 181 chemin du Lazaret, 06230 Villefranche-sur-mer, France.

Corresponding author: Leclère, Lucas (leclere@obs-vlfr.fr).

Abstract

The fresh water polyp *Hydra* provides textbook experimental demonstration of positional information gradients and regeneration processes. Developmental biologists are thus familiar with *Hydra*, but may not appreciate that it is a relatively simple member of the Hydrozoa, a group of mostly marine cnidarians with complex and diverse life cycles, exhibiting extensive phenotypic plasticity and regenerative capabilities. Hydrozoan species offer extensive opportunities to address many developmental mechanisms relevant across the animal kingdom. Here we review recent work from non-*Hydra* hydrozoans – hydromedusae, hydroids and siphonophores – shedding light on mechanisms of oogenesis, embryonic patterning, allorecognition, stem cell regulation and regeneration. We also highlight potential research directions in which hydrozoan diversity can illuminate the evolution of developmental processes at micro- and macro-evolutionary time scales.

Introduction

Although the relationships between non-bilaterian animals are currently somewhat controversial, the position of cnidarians as the closest relatives of bilaterians seems relatively undisputed. This has helped to establish the anthozoan cnidarian *Nematostella vectensis* as a key model for molecular studies in evolutionary aspects of developmental biology. Anthozoa is one side of the deepest split in the cnidarian lineage, the other being Medusozoa, a group united by the presence of a medusa (*i.e.* a jellyfish) stage in their life-cycle. Medusae show high morphological complexity, including features such as striated muscle and elaborate sensory structures, and in some instances even camera eyes. Medusozoa is composed of four clades: Hydrozoa, Staurozoa, Cubozoa and Scyphozoa (Figure 1a) [1•]. Hydrozoa is the biggest and most diverse of these, comprising more than 3500 species.

Detailed observation and experimentation on marine hydrozoan species fueled many discoveries and conceptual advances in the second half of the 19th century, relating notably to germ plasm theory, evolution of germ layers, and regeneration. Weismann, Haeckel, Morgan, Huxley, Driesch, the Hertwig brothers and Metchnikov all worked extensively on marine hydroids and hydromedusae. During the 20th century, studies in the freshwater polyp *Hydra* dominated hydrozoan research in cell and developmental biology, resulting in important findings related to organizer activity, morphogen gradients, pluripotent stem cells, and more recently, evolution of aging (*e.g.* [2]) and tumorigenesis (*e.g.* [3]), and interactions between animals, symbionts and microbiota (see [4]). *Hydra* is still, at the time of writing, the only hydrozoan for which a genome sequence has been published [5]. Several excellent recent reviews have covered *Hydra* research (see [4,6–12•]) and so here, we will focus on current work on other hydrozoan species to highlight their contribution and potential for understanding fundamental developmental processes and evolutionary questions. Unlike *Hydra*, some of these species allow easy experimental access to sexual reproduction and embryonic development.

In recent years, marine hydrozoan species have been used increasingly for molecular studies, notably *Clytia hemisphaerica* [13], *Hydractinia echinata* and its close American relative *Hydractinia symbiolongicarpus* [14], *Cladonema radiatum* [15] and *Podocoryna carnea* [16]. Improved molecular technologies and decreasing sequencing costs are aiding emergence of additional models such as *Turritopsis dohrnii* [17,18], *Eleutheria dichotoma* [19,20] and the siphonophore *Nanomia bijuga* [21,22•,23] (summarized in Table 1).

Phenotypic plasticity and life cycle complexity

The typical hydrozoan life cycle comprises three basic forms: the motile 'planula' larva, sessile polyps (which typically propagate to form extensive colonies by asexual reproduction), and the pelagic medusa, a sexual form usually generated by budding from a polyp (in contrast to the scyphozoan medusa, which forms by a process known as strobilation [24••]). The evolutionary plasticity of this presumably ancestral program is remarkable, as illustrated by the loss of the medusa in the lineages leading to *Hydra* and *Hydractinia*. Molecular based phylogenies imply more than 50 independent losses of the medusa and at least 2 losses of the polyp within Hydrozoa, and show that major changes in life cycles and in colonial organization have occurred over short evolutionary time-scales [25–35•] (Figure 1b-c). Hydrozoan life cycles can include sexual and asexual reproduction at almost any stage, and the transitions can occur in atypical sequences, such as the famous medusa to polyp transformation ('reverse development') of *Turritopsis dohrnii* [17]. Determining the molecular mechanisms (including epigenetic modifications and response to environmental factors), and evolutionary processes triggering morphological and life cycle plasticity are important challenges for future research [36]. The history of genes expressed preferentially in the medusa (relating for instance to striated muscle or sense organs) has started to be addressed [37,38], and the first comparisons of transcriptomes from *Podocoryna* and *Hydractinia*, related species with and without medusae respectively, have been made [39•]. A successful approach that allowed identification of cnidarian-specific genes regulating tentacle patterning in *Hydra* involved subtractive hybridization between cDNAs from morphologically distinct *Hydra* strains, followed by functional validation *via* transgenesis [40].

Additional life cycle complexity in hydrozoans arises *via* the formation in many species of complex benthic or pelagic colonies comprising connected polyps specialized for different functions (*e.g.* budding, feeding, defense). The siphonophores are pelagic colonies which have attained extreme complexity including many different polyp types, but also specialized attached medusa forms serving distinct functions (*e.g.* reproduction, swimming) [21,22•,41]. Very little is known about how the polyp and medusa components of complex colonies acquire different forms and functions during development and evolution, although the advent of molecular analysis in siphonophores is promising [22•,23]. New transcriptomic data for several polyp types in *Hydractinia* [42–44] provide many candidates for future investigations of the developmental mechanisms leading to a division of labor between polyps within a colony (see Table 1).

In some hydrozoans groups, polyp colonies of the same species in close contact can fuse or reject depending on how closely genetically related they are. This phenomenon has been successfully

dissected at the molecular level in *Hydractinia*, starting with the seminal work of von Hauenschild in the 1950s (see [45]). A long and outstanding genetic investigation, involving crosses of many related and unrelated strains [46], led to the discovery of an allorecognition complex composed of two genetic loci, alr1 [47] and alr2 [48]. The Alr1 and Alr2 proteins show huge diversity in natural populations [49••,50]. They have recently been shown to be homophilic ligands binding only to themselves [51••]. In natural populations additional genetic factors may also be involved in the allorecognition process [52].

Allorecognition regulation has important consequences for individual and colonial biology and genotype survival. Fusion between sexually generated polyps has recently being proposed as the mode of colony formation in the genus *Ectopleura* [35•]. Rejection between colonies can induce necrosis in *Hydractinia* [53]. Contact between genetically distinct colonies results in mixing of stem cells, which can lead to invasion, takeover and eventually changes in morphology. A fascinating corollary is that evolutionary selection in this context might act at the level of the stem cells, as also proposed in the colonial ascidian *Botryllus* [54].

Medusa evolution and development

Cnidarians are often represented as having a simple morphology and relatively few cell types, reflecting the situation in the common bilaterian-cnidarian ancestor. The medusa form, however, has a sophisticated organization, including specialized sensory and reproductive organs and the fast-contracting striated muscle of the bell for swimming [15,13,55] (Figure 2a), providing an opportunity to compare the developmental and evolutionary origins of these structures in cnidarians and bilaterians. Molecular studies of hydrozoan medusae sensory structures have so far focused mainly on light detection. Eyes of *Cladonema* medusae express Opsin photoproteins and Six/Pax/Eya transcription factors [15,56,57], suggesting a common origin of the molecular mechanisms of eye development and convergence of the eyes as complex structures between Hydrozoa and Bilateria.

The medusa stage itself has been the subject of historical dispute, with opposing camps regarding it as a primitive cnidarian trait lost in Anthozoa, or a derived feature of Medusozoa. From a phylogenetic perspective, recent debate has revolved around potential paraphyly of Anthozoa and the position of Staurozoa (so-called ‘stalked jellyfish’). It is now widely accepted that the medusa is a derived character of Medusozoa, although the most recent phylogeny recovers monophyletic Anthozoa, as sister to Medusozoa, with Hydrozoa (rather than Staurozoa) as sister group to the other medusozoans [1•].

Gene expression data relating to medusa development is accumulating gradually (*e.g.* [58–61•]), but remains scarce compared to planula and polyp data, while gene function studies in the medusa are still largely lacking [20]. Detailed comparative anatomical, molecular and functional studies between medusae of the different medusozoan groups will be insightful for deciphering the origin(s) of the medusa form. The evolutionary origin of hydrozoan striated muscle, traditionally considered homologous between cnidarians and bilaterians [62], was addressed by Steinmetz and coworkers [63••] (Figure 2a). Genomic analyses and *in situ* hybridization approaches showed that key bilaterian striated muscle proteins, notably components of the Z-disk, are not present in *Clytia*, while most of the bilaterian striated muscle genes are either expressed broadly or in other cell types. This study thus provides compelling evidence for convergent evolution of striated muscle in hydrozoans and bilaterians.

Gametogenesis and spawning

The transparency and accessibility of many hydrozoan sexual forms makes them particularly good models for studying the widely conserved regulatory mechanisms of animal gametogenesis, and dissecting the mechanisms of oocyte maturation, spawning and fertilization (discussed in detail in [64], Figure 2b). Studies in several medusae including *Cladonema, Cytaeis* and *Clytia* are allowing analysis of successive regulatory steps. For technical reasons (notably access to microinjection of Morpholinos/mRNAs/indicators), the main research focus so far has been on the fully grown oocyte and spawned egg. Spawning in both males and females is triggered by light, which induces somatic cells of the gonad to secrete a small diffusible hormone that awakens the dormant gametes. Recent studies suggest that neuropeptide-related molecules participate in this process [65]. The essential downstream response of oocytes to the hormone is an immediate rise in cytoplasmic cAMP levels and PKA activation [66]. This stimulates both release of meiotic arrest and entry into the meiotic divisions and parallel activation of the Mos-MAP kinase pathway responsible for regulation of oocyte-specific processes such as polar body formation and post-meiotic cell cycle arrest [67]. MAP kinase pathway inactivation following fertilization, along with cytoplasmic Ca^{++} release, is also involved in regulating polyspermy [68•]. Concerning the regulation of early steps of gametogenesis such as entry into meiosis and oocyte growth, which represent major bottlenecks in research in reproductive biology, the advent of gene editing techniques is opening the powerful possibility of combining gene function analysis and imaging using transparent hydrozoan gonads.

Body axis evolution and development

Studying axis formation in a range of cnidarians, including hydrozoans, offers an informative perspective for the understanding of body plan evolution in metazoans. It is still debated whether the oral-aboral axis of cnidarians is homologous to any of the body axes of bilaterians, but it seems very likely that localized Wnt signaling was a feature of the gastrulation pole of the last common ancestor of these major animal groups.

Each hydrozoan form, planula, polyp and medusa, is organized with respect to a principal axis of polarity termed oral-aboral (Figure 3a). For instance the planula larva has an aboral specialization of the nervous system [69], and the medusa has integrating nerve rings around the bell margin [55]. Key to establishment and maintenance of this axis is the oral pole organizer. The key molecular pathway of oral organizer activity in *Hydra* is Wnt/β-catenin signaling [11], activated by the diffusible ligand Wnt3 and maintained at the oral tip by a complex auto-feedback loop [70]. Oral organizers have now been described at the oral pole of the planula larvae and polyp in several marine hydrozoan species [71,72], with the Wnt/β-catenin pathway established as a key player in the regulation of the planula and polyp organizer.

In *Clytia* larvae, functional studies involving morpholino and mRNA injection into the egg have shown that axis formation is initiated by maternal mRNAs coding for the ligand Wnt3 (Figure 3a, b), and for two Frizzled family receptors [73,74] which become localized in the egg during oocyte growth and maturation [75]. During early development, Wnt3 and Fz1 together activate the Wnt/β-catenin pathway at the oral pole, while Fz3 acts as a negative regulator at the aboral pole. In parallel, the transmembrane protein Strabismus interacts with Fz1 across neighboring cell boundaries to direct morphogenesis along the oral-aboral axis *via* a typical PCP mechanism [76••]. PCP coordinates the alignment of the ectodermal cilia to ensure unidirectional swimming, and orients cell movements participating in elongation of the developing larva (Figure 3b). Common involvement of Frizzled proteins in establishing a Wnt/β-catenin activity gradient and PCP is likely to be an ancestral mechanisms to couple morphological and molecular polarity during development [76••]. A transcriptomic study identified potential Wnt/β-catenin downstream targets and genes regulated by the PCP pathways [77••], providing several promising candidates for roles in organizer activity.

Analyses of Wnt pathway regulation in *Hydractinia* have focused mainly on the polyp. Wnt3 signaling *via* a TCF transcription factor induces development of oral structures [78]. Other studies in *Hydractinia* have addressed the dramatic metamorphosis that transforms the planula into the polyp,

and notably the extensive caspase dependent apoptosis at the oral pole during metamorphosis [79–82]. This apoptosis is essential for the formation of the primary polyp [83], playing a constructive role [81,84] as it does during regeneration in *Hydra* [85]. Interestingly, *Wnt5a* expressing cells at the oral pole of *Hydractinia* larvae appear to be protected from apoptosis during metamorphosis [71].

Cell type plasticity and stem cell systems in Hydrozoa

Hydrozoans characteristically exhibit a very strong ability to regenerate one or all their phenotypic forms, a capacity underpinned by extreme cell type plasticity. This includes *bone fide* transdifferentiation, as demonstrated using *Podocoryna carnea* by Schmid and collaborators, who convincingly showed transdifferentiation of striated muscle cells isolated from the medusa into several cell types including smooth muscle, sensory cells, gland cells and nematocytes (stinging cells characteristic of Cnidaria) [16].

Hydrozoan regeneration involves active stem cell systems, first characterized in *Hydra*, where three types of stem cell coexist: ectodermal epithelial stem cells, endodermal epithelial stem cells and 'insterstitial' cells (i-cells) (see [7]) (Figure 3c). The first two basic epithelial cell types support the constant renewal of the ectodermal and endodermal epithelia. I-cells are a hydrozoan-specific type of undifferentiated multipotent stem cell that can differentiate into nerve cells, nematocytes, gland cells and also germ cells. I-cell developmental potential varies between species. Thus, in *Hydractinia echinata*, i-cells can differentiate not only into nerve cells and nematocytes, but also into epithelial cells, as demonstrated using mutants, BrdU labeling and grafting experiments [86], and confirmed using transgenic tools [87] (Figure 3c).

Progress in understanding the molecular regulation and cellular dynamics of hydrozoan stem cells was greatly accelerated by the development of transgenic lines both in *Hydractinia* [88••] and *Hydra* [89], which allowed tracking of stem cells *in vivo*, notably during regeneration [88••] (Figure 3d). Pioneering studies in *Hydra* have compared molecular signatures of different cell types by transcriptome analysis of fluorescence-tagged sorted cells from transgenic lines [90] and established proteomic methods [91••]. In *Hydractinia* a POU-domain containing transcription factor of the class 3/5 involved in regulating pluripotency has been identified [92•], of particular interest given the role of the (class 5) POU-domain factor Oct 4 in mammals. Similarly, in *Clytia*, several Sox family transcription factors (groups B, C, E and F) were found expressed either in stem-cells or differentiated cells, suggesting a regulatory role in multipotency maintenance operating widely across the animal kingdom [93]. A recent study has shown a correlation between nuclear localization

of the Hippo pathway component Yorkie and cell division in *Clytia* medusae [94], suggesting that regulation of cell proliferation by this transcriptional cofactor is conserved between Cnidaria and Bilateria.

I-cells in *Clytia* [95,96•] and *Hydractinia* [88••,92•,97,98], as in *Hydra* (*e.g.* [90,99]), also express orthologues of genes typically associated with germ line or stem cell function such as *Piwi*, *Nanos1*, and *Vasa*. I-cell formation during embryogenesis was addressed in *Clytia,* and appears to involve both determinant inheritance and inductive mechanisms [96•]. I-cells originate at the onset of gastrulation in a region inheriting localized *Piwi*, *Nanos1*, *Vasa* and *Pl10* mRNAs from the animal pole of the egg, evoking the 'preformation' mode of germ-line specification by maternal 'germ plasm' well known in insects and frog embryos. I-cells can also form in *Clytia* planulae in the absence of the key egg animal pole region, implying the involvement of inductive mechanisms [96•].

Conclusion

Hydrozoans have much to contribute to our understanding of basic developmental and cell biology processes and their evolution, as well as to relatively neglected areas of biological complexity such as colony level organization and asexual reproduction. Rapid technological advances mean that it is now feasible with quite modest budgets to undertake genome sequencing, large scale gene expression/transcriptomics studies, functional analyses at all life cycle stages *via* gene editing, as well as cell/molecule tracking using transgenic lines carrying diverse fluorescent reporters. With these accumulating resources, we expect in the next few years that a wide range of marine hydrozoan species will emerge as valuable experimental models.

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• The first functional study of an i-cell expressed gene in *Hydractinia*, the POU-domain protein Pln. Strikingly, forced Pln expression in epithelial cells induces stem cell neoplasms, while RNAi-mediated Pln downregulation promotes differentiation. It is tempting to draw parallels with the stem cell regulatory role of mammalian Oct4, although existing molecular phylogenies do not seem to position these two transcription factors as closely related within the large POU-domain family.

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• The multiple fate possibilities of i-cells include germ cells, so these hydrozoan stem cells can in some ways be equated with the fate-restricted 'germ lines' of many bilaterians. This parallel was thrown into the spotlight by the discovery in *Clytia* of 'germ plasm' containing a shared set of maternal mRNAs, which appears to be inherited by i-cell precursors. How i-cell fate is specified remains to be resolved, since *Clytia* embryo fragments with or without germ plasm can develop i-cells.

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Figure legends

Figure 1. Hydrozoan phylogeny and life cycle diversity and evolution. (a) Phylogeny of Cnidaria following [1•], with Bilateria as the outgroup. Hydrozoa is sister to the other medusozoans (Staurozoa, Scyphozoa and Cubozoa). Red, orange and blue dots indicate presence of planula, polyp or medusa stages respectively in the last common ancestor of these groups. Acquisitions of the medusa and polyp stages are indicated by orange and blue diamonds respectively. Staurozoa adult stages show a mix of polyp and medusa characters. **(b)** Phylogeny and evolution of Hydrozoa following [32]. The groups 'Filifera', 'Trachymedusae' and 'Limnomedusae' are probably not monophyletic [30, 32]. Losses of the polyp stage in the clade Trachylina are indicated by white diamonds. The planula stage was likely lost in the common ancestor of Aplanulata [35•]. It is still equivocal whether the medusa stage was lost concomitantly, or later, within this clade [35•]. Mature siphonophores are composed of specialized polyps and medusae that remain attached within the colony. **(c)** Examples of the four main life cycle types found in Hydrozoa. In all cases sexual reproduction generates planula larvae. **(i)** *Clytia hemisphaerica;* polyps develop from planulae by metamorphosis; medusae by budding from polyps. Two polyp types co-exist within the colony, specialized for medusa budding (gonozoid; left) and feeding (gastrozoid; right). Image from [13]. **(ii)** *Aglantha digitale;* the planula larva metamorphoses directly into a medusa. **(iii)** *Hydractinia echinata;* The colony contains four types of polyp (from left to right): gonozooid, gastrozooid, dactylozooid, tentaculozooid. The common part of the colony also includes protective spines. **(iv)** *Nanomia bijuga;* the larva develops into a complex polygastric colony comprising different types of medusa- (*e.g.* nectophores, propelling the colony – in blue) and polyp- (*e.g.* gastrozooid – in orange) derived structures, distributed in ordered arrays. Image in (c-i) reproduced from [13]. Images courtesy of Alexander Semenov (c-ii), Uri Frank (c-iii), Brad Gemmell (c-iv).

Figure 2. *Clytia* medusa: muscle and egg formation. (a) Medusa organization and development**. (i)** Diagram of a mature *Clytia* jellyfish; cc: circular canal, ex-u: ex-umbrella, go: Gonad, ma: manubrium tb: tentacle bulb, te: tentacle, rc: radial canal, sub-u: sub-umbrella surface, ve: velum. **(ii)** Convergent evolution of striated muscles in Bilateria and Cnidaria [63••]. **(iii)** Subumbrellar striated muscle of a young medusa stained with phalloidin (red in iii and iv; grey in v and vi) and nuclei stained with Hoechst (blue). **(iv)** Newly budded medusa – at this stage the striated muscle layer (sm) covers most of the sub-umbrella surface and is sandwiched between an endodermal (end) and ectodermal smooth muscle (ect) layers. gp: gonad primordium, other labels as in (i). Image courtesy of Johanna Kraus [61•]. **(v)** Confocal section of a medusa bud within the gonozoid. Prominent features are the

developing manubrium (ma), striated muscles (sm) and tentacle bulbs (tb). **(vi)** Confocal section of a an early medusa bud composed of ectoderm (ect), endoderm (end), and a third layer, the entocodon (ent), which generates the striated muscle of the medusa. The entocodon is a unique feature of hydrozoan medusae; homology to mesoderm has been proposed but is not widely accepted (see [62]). **(b)** *Clytia* oocyte maturation [64, 67, 75]. **(i)** Female gonad containing fully grown oocyte ready to mature (FGO). **(ii)** Fully-grown oocyte with germinal vesicle (GV) close to the cell surface on the ectoderm side (ect), opposite the contacts with endodermal cells (end). **(iii)** The same oocyte about 15 min after a light cue, undergoing germinal vesicle breakdown (GVBD). A light cue initiates the release of maturating inducing factor (MIH) by the gonad ectoderm, which unblocks the oocyte's meiotic arrest at prophase I *via* an cytoplasmic cAMP release / PKA activation, leading to activation both of MPF and of the Mos-MAPK pathway. **(iv)** Spawning of the same oocyte, about 100 min after light; involves rupture of the ectodermal epithelium just after second polar body (pb) emission. **(v)** *Clytia* egg with the heads of attracted sperm (sp) visible in the surrounding jelly. Fertilization induces MAPK inactivation and increase of intracellular calcium, shown in *Cytaeis* to prevent polyspermy [68•]. Scales bars: 100 µm, except for (a-iii): 10 µm.

Figure 3. Embryonic axis establishment and stem cell plasticity. (a) Schematic representation of *Wnt3* expression at five embryonic stages in *Clytia*. From left to right: fertilized egg, blastula, early gastrula, late planula, primary polyp. *Wnt3* mRNA is localized at the egg animal pole, and oral (top in all figure parts) expression is maintained at all stages. Drawings modified from [13]. **(b)** *Wnt3* Morpholino injected embryos show no oral-aboral polarity. Images reproduced from [74]. *Strabismus* Morpholino larvae show disrupted PCP and ciliogenesis. Images reproduced from [76••]. Arrowheads indicate microvilli marking sites where ciliogenesis has failed. **(c)** Schematic representation of cell lineages in *Hydra* (top) and *Hydractinia* (bottom). Epithelial cells (EC) can be generated from interstitial stem cells (i-cells) in *Hydractinia*, but not in *Hydra*. Intermediate differentiation stages are not represented [86]. **(d)** Summary of *Hydractinia* polyp head regeneration. Proliferating i-cells (blue dots) migrate towards the oral pole after bisection and participate in the formation of the new head structures. Tools routinely used in *Hydractinia* regeneration studies include stable transgenic fluorescent reporter lines and proliferation assays (BrDU). Images from [88••]. Scales bars: 100 µm, except the four right panels in (b): 5 µm.

Table 1 – Hydrozoans used for genetic/molecular studies

Clade/Species	Available techniques	Main research topics
Aplanulata		
Hydra spp.	*In situ* hybridization; RNAi; egg micro-injection; transgenesis; mutagenesis; proteomic; RNAseq of fluorescent-tagged sorted cells	Stem cell; regeneration; pattern formation; organizer; budding; symbiosis; sex determination; aging [2-12,40,70, 85,89-91,99]
Capitata		
Cladonema radiatum	*In situ* hybridization	Specification of sensory cells; eye development and regeneration [15,56,57]
Eleutheria dichotoma	*In situ* hybridization; RNAi	Hox genes; patterning [19,20]
Filifera		
Cytaeis uchidae	Manipulation of oocytes and eggs	Reproductive biology [65,66,68]
Hydractinia echinata	*In situ* hybridization; RNAi, Morpholino and mRNA injection; transgenesis	Polyp regeneration; stem cells; colony development [14,42,71,78–83,86–88,92,97,98]
H. symbiolongicarpus	*In situ* hybridization; genetics	Allorecognition; polyp polymorphism [14,39,43-53]
Podocoryna carnea	*In situ* hybridization; manipulation of medusa tissues	Colony development; medusa development; muscle transdifferentiation [16,17,38,60]
Turritopsis dohrnii	'reverse development'	Life cycle plasticity [17,18]
Leptothecata		
Clytia hemisphaerica	*In situ* hybridization; Morpholino and mRNA injection; manipulation of oocytes and eggs; CRISPR and TALEN gene editing (unpublished)	Larval development; polarity establishment and maintenance; stem cells; oocytes; muscle evolution; medusa formation [13,58,61,63,64,67,73-77,93-96,100]
Siphonophorae		
Nanomia bijuga	*In situ* hybridization	Colony development [21-23,27,101]

Note: development of extensive transcriptome and genome resources are underway for most of these species. *In situ* hybridization technique and/or transcriptome resources have been developed for a few others species (*e.g.* [1,37,59]).

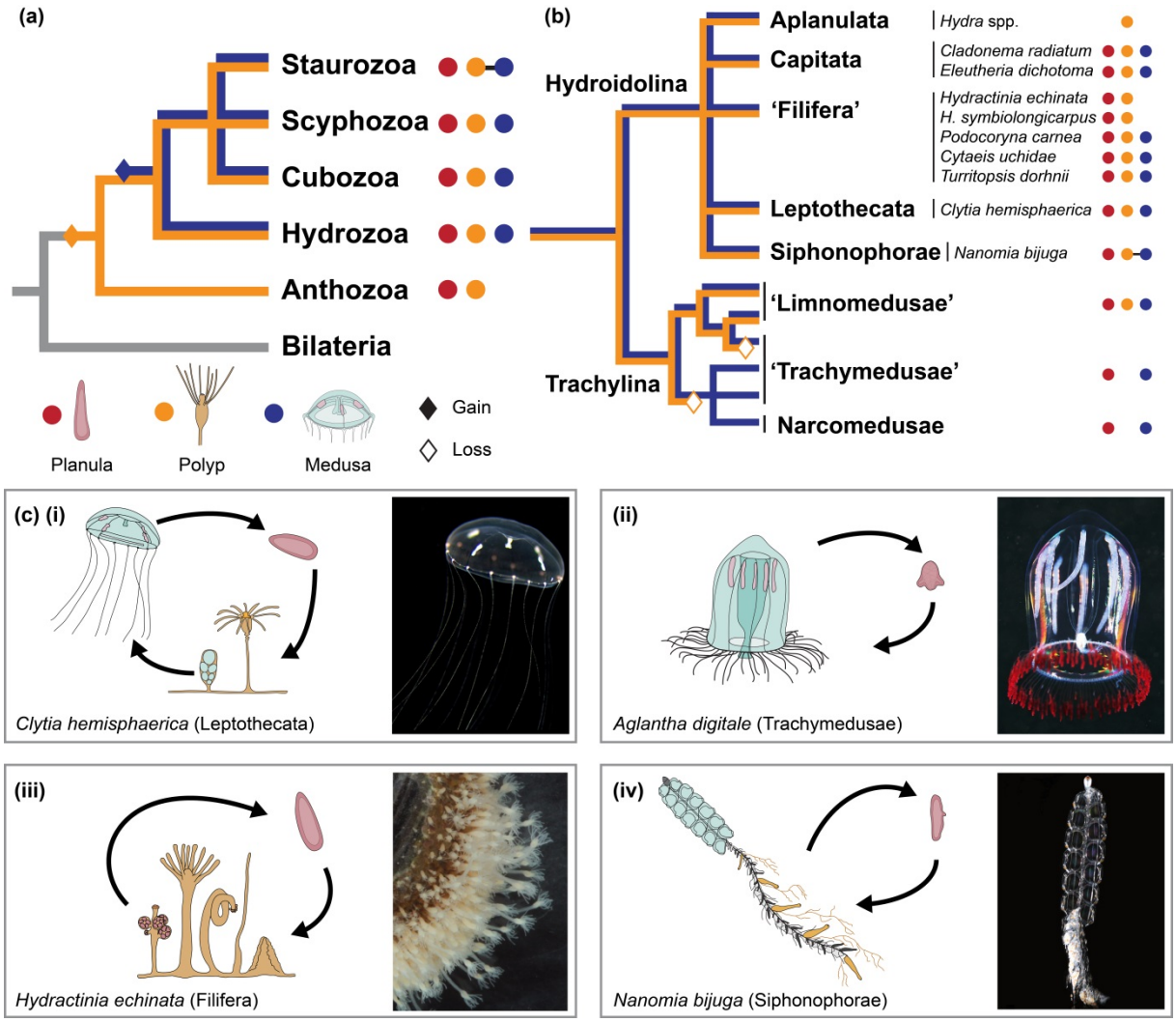
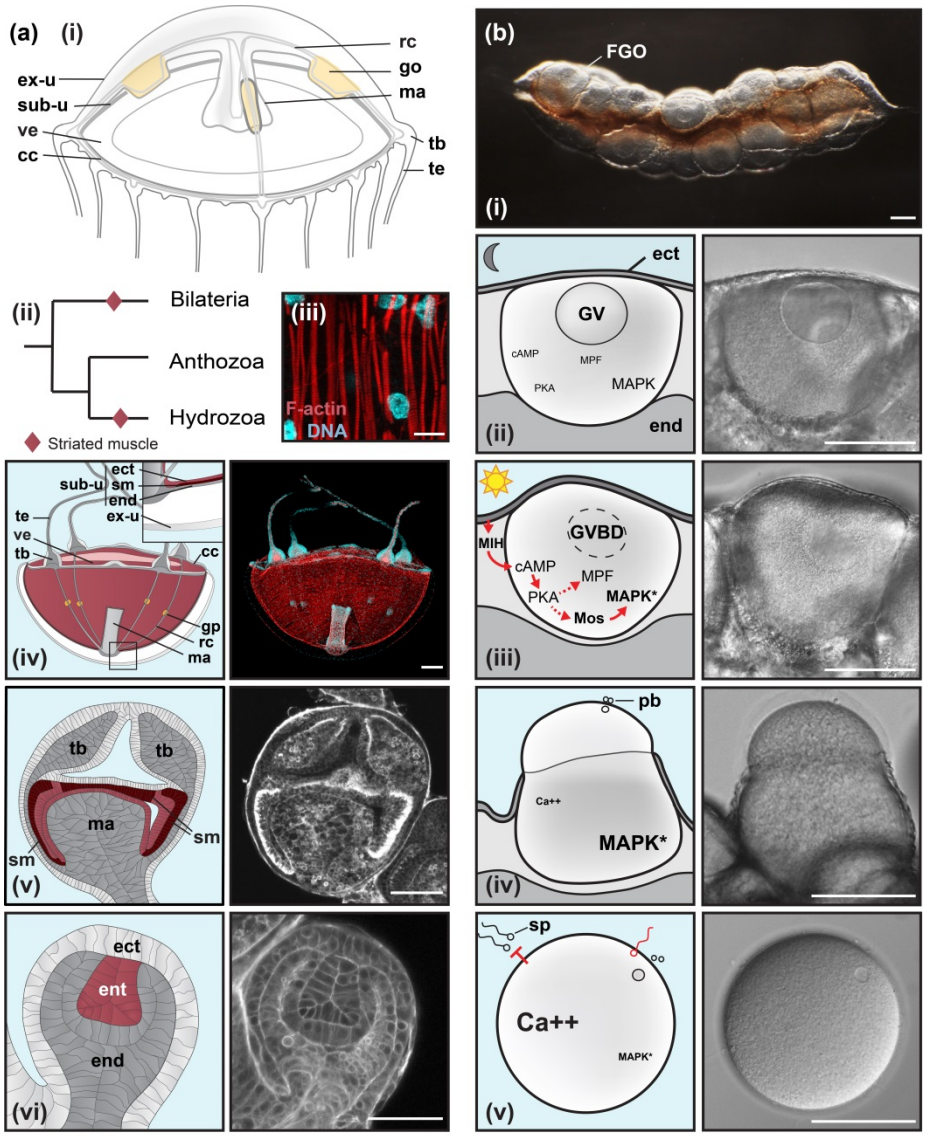

(a)
Staurozoa
Scyphozoa
Cubozoa
Hydrozoa
Anthozoa
Bilateria
Planula
Polyp
Medusa
Gain
Loss
(b)
Hydroidolina
Trachylina
Aplanulata
Capitata
'Filifera'
Leptothecata
Siphonophorae
'Limnomedusae'
'Trachymedusae'
Narcomedusae
Hydra spp.
Cladonema radiatum
Eleutheria dichotoma
Hydractinia echinata
H. symbiolongicarpus
Podocoryna carnea
Cytaeis uchidae
Turritopsis dorhnii
Clytia hemisphaerica
Nanomia bijuga
(c) (i)
Clytia hemisphaerica (Leptothecata)
(ii)
Aglantha digitale (Trachymedusae)
(iii)
Hydractinia echinata (Filifera)
(iv)
Nanomia bijuga (Siphonophorae)

Figure 1


(a) (i)
ex-u
sub-u
ve
cc
rc
go
ma
tb
te
(ii)
Bilateria
Anthozoa
Hydrozoa
Striated muscle
(iii)
F-actin
DNA
ect
sub-u
sm
end
ex-u
te
ve
tb
cc
gp
rc
ma
(iv)
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tb
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sm
(v)
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(vi)
(b)
FGO
(i)
ect
GV
cAMP
MPF
PKA
MAPK
(ii)
end
GVBD
MIH
cAMP
MPF
PKA
MAPK*
Mos
(iii)
pb
Ca++
MAPK*
(iv)
sp
Ca++
MAPK*
(v)

Figure 2

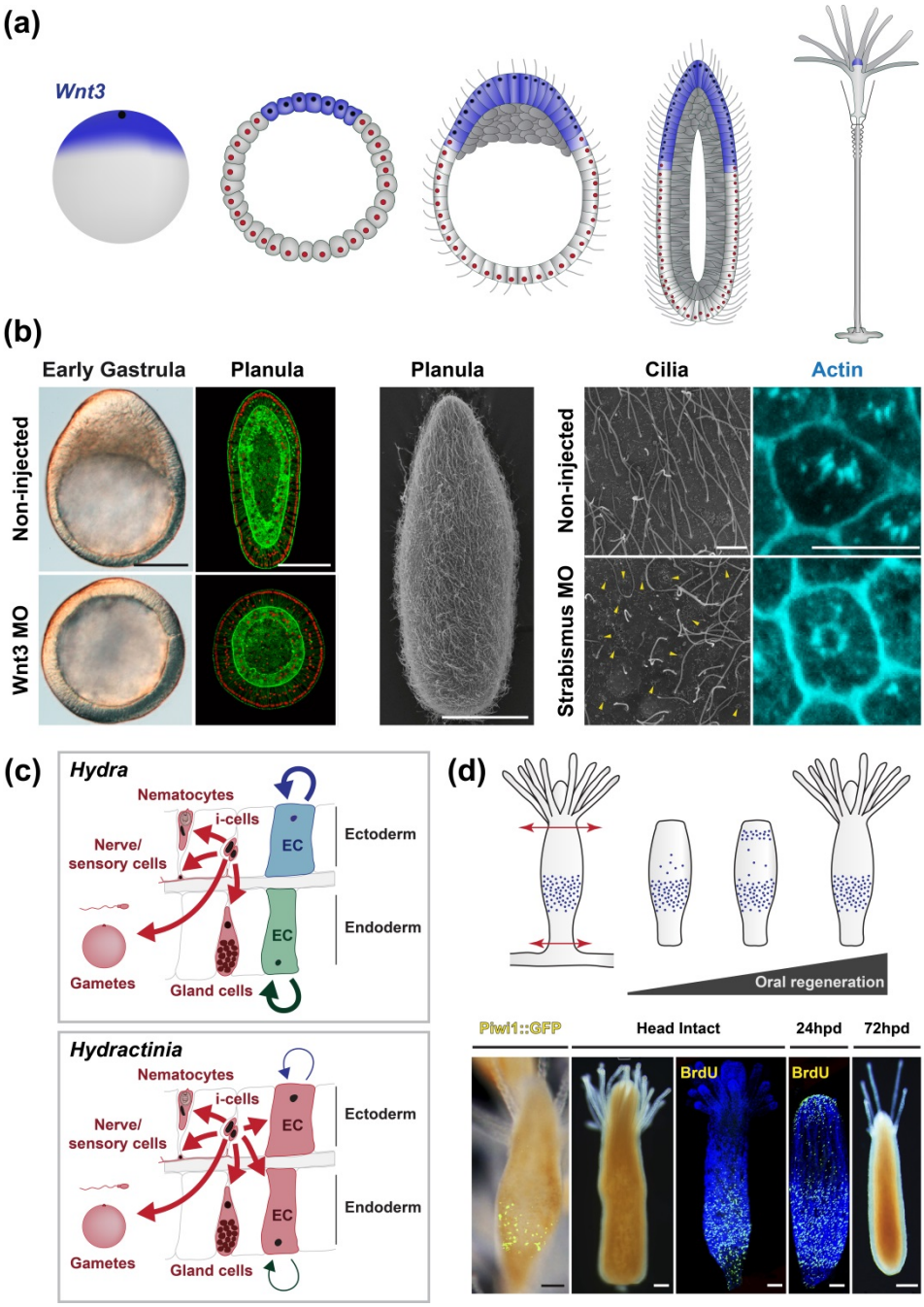
(a)
Wnt3
(b)
Early Gastrula
Planula
Planula
Cilia
Actin
Non-injected
Wnt3 MO
Non-injected
Strabismus MO
(c)
Hydra
Nematocytes
i-cells
Nerve/
sensory cells
EC
Ectoderm
EC
Endoderm
Gametes
Gland cells
Hydractinia
Nematocytes
i-cells
Nerve/
sensory cells
EC
Ectoderm
EC
Endoderm
Gametes
Gland cells
(d)
Oral regeneration
Piwi1::GFP
Head Intact
24hpd
72hpd
BrdU
BrdU

Figure 3