Review

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Clytia hemisphaerica: a jellyfish cousin joins the laboratory

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***Clytia hemisphaerica*, a member of the early-branching animal phylum Cnidaria, is emerging rapidly as an experimental model for studies in developmental biology and evolution. Unlike the two existing genome-sequenced cnidarian models *Nematostella* and *Hydra*, *Clytia* has a free-swimming jellyfish form, which like ''higher'' animals (the Bilateria) has a complex organization including striated musculature, specialized nervous system and structured sensory and reproductive organs. *Clytia* has proved well suited to laboratory culture and to gene function analysis during early development. Initial studies have shed light on the origins of embryonic polarity and of the nematocyte as a specialized neurosensory cell, and on the regulation of oocyte maturation. With a full genome sequence soon to become available, and a clear potential for genetic approaches, *Clytia* is well placed to provide invaluable information on core mechanisms in cell and developmental biology, and on the evolution of key features of animal body plans.**

Clytia joins the laboratory

A small but influential cast of model species, adopted by biologists for their suitability for genetic and experimental approaches, has made an enormous contribution to our current understanding of embryonic development and other essential biological processes. From a phylogenetic point of view, however, this cast is rather limited: many branches of the animal kingdom are missing and several of the popular models (for instance the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans)* are now acknowledged as atypical fast-evolving or 'derived' representatives of their respective phyla. Recent years have thus seen an accelerating effort to increase the evolutionary range of model organisms available for functional studies. This effort has been greatly facilitated by the downwardly spiralling costs of transcriptome and genome sequencing, once a prohibitive factor for establishing non-standard models. In this review, we introduce *Clytia hemisphaerica,* an up-and-coming model species from the early branching and highly diverse phylum Cnidaria. This species, which is typical of the Hydrozoa class in having a free-swimming jellyfish form as well as a vegetatively propagating polyp (see below), was chosen about five years ago as a promising candidate model for molecular and cellular studies in development and evolution on the basis of its phylogenetic position, its favourable characteristics for laboratory culture, and the accessibility to experimental manipulation and microscopy of its oocytes, eggs, embryos and larvae.

A cnidarian model with a 'complete' life cycle

The Cnidaria are divided into two evolutionary branches: the Anthozoa (benthic polyp animals including sea anemones and corals); and the Medusozoa, which typically have free-swimming jellyfish forms as well as benthic polyps in their life cycles. The traditionally recognised Medusozoa classes are the Hydrozoa, Cubozoa and Scyphozoa, with the Staurozoa (benthic cnidarians with a mixture of polyp and medusa characters) now recognised as a fourth class (Figure 1).

Cnidarians are morphologically simple, containing relatively few cell types and lacking elaborate organ structures. The cnidarian ancestor diverged early during animal evolution from that of the bilaterian animals (comprising the protostomes such as *Drosophil*a and *Caenorhabditis* and deuterostomes such as sea urchins and vertebrates). Somewhat counter-intuitively, cnidarians have been found to possess virtually all the families of regulatory developmental genes characterized in bilaterians [1–3] with the diversification of these families largely equivalent to that seen in bilaterians [4–7]. In other words, evolution has generated a huge range of morphologies separately in these two major animal clades, using the same pre-existing tool kit of genetic regulators. Comparing distant cousins within and between these groups can thus help to identify ancestral mechanisms and to inform us about the routes to animal diversity.

In the genomic era, two cnidarian model species are at the forefront and have completed genome sequences: the starlet sea anemone *Nematostella vectensis* [3,8], a member of the class Anthozoa, and the freshwater polyp *Hydra* [9] from the class Hydrozoa. *Clytia* genome sequencing is underway (Box 1), and genomes of other cnidarians from different branches will undoubtedly follow [10], opening the way to the comparative studies necessary to fully understand cnidarian development and its evolution.

The main particularity of the *Clytia* model with respect to *Nematostella* and *Hydra* is that it has a 'complete' life cycle, alternating between vegetatively propagating polyps and a sexual free-swimming jellyfish (medusa) form (Figure 2). It is now generally believed that the single-

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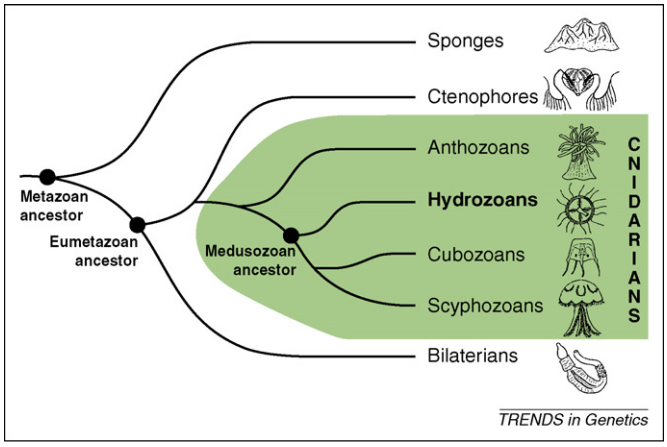

Figure 1. The position of hydrozoans such as *Clytia* in the animal tree of life. This simplified tree summarizes the relationships among early-diverging metazoan phyla as obtained in a recent phylogenomic study [75]. Cnidarians and ctenophores are therefore shown as a monophyletic group (the traditional Coelenterata), although this remains to be confirmed [78,79]. The cnidarian classes Cubozoa, Scyphozoa and Hydrozoa, together with the Staurozoa (omitted here due to their uncertain position), form the Medusozoa branch. The tiny phylum Placozoa has been omitted because its position is contentious [80,75,81]. Within Medusozoa, the medusa form may have arisen once or several times [11].

phase life cycle of anthozoans was the ancestral state in the Cnidaria, with the medusa form having been inserted into the life cycle either once or several times in the Medusozoa branch [11] (see Figure 1 in Ref. [12] in this issue). *Hydra* is an atypical hydrozoan, having adopted a freshwater habitat and secondarily lost the medusa stage [13]. Other hydrozoans used for molecular and/or developmental studies include the marine colonial polyp *Hydractinia* [14–16], and the complete life cycle species *Podocoryne* [17,18] and *Cladonema* [19,20].

In general, cnidarian medusae show significantly more anatomical complexity than polyp forms, including smooth and striated musculature for rapid swimming and a well-organized nervous system integrating specialized balance organs (statocysts), and even eyes in some species (including *Cladonema*). The relatively simple nervous systems of *Nematostella* and *Hydra* polyps comprise dispersed nerve nets locally condensed into nerve rings, notably around the mouth [21–23]. Hydrozoan medusae, including *Clytia,* show a higher degree of specialization, with two parallel condensed nerve rings running round the bell periphery, one (outer ring) organized for integrating sensory inputs and the other (inner ring) for coordinating motor responses (Figure 3) [24,25].

Given the presence of a medusa stage, *Clytia* should be able to teach us much about the evolution of complex morphological traits, as well as how different phenotypes (polyp versus medusa) can be produced with the same genome. A preliminary comparison of the current *Clytia* transcriptomic dataset (Box 1) with *Nematostella* and *Hydra* genome data has revealed the potential importance in this context of selective gene retention from the common ancestor, in addition to the acquisition of new genes [12].

Characteristics of *Clytia* as a laboratory animal

Clytia species, formerly described under a variety of genus names, including *Phialidium* and *Campanularia*, are abundant worldwide. Owing to their easy availability and convenience for laboratory manipulation, they have been the material of choice over the years for research in a wide range of domains, including fertilization, regeneration, ecology and physiology, as well as the characterization of GFP and Aequorin family proteins [26–33]. They played a particularly important part in the history of cnidarian developmental biology, with the American species *Clytia gregaria (Phialidium gregarium)* providing

Box 1. Available and upcoming *Clytia* resources

Living resources

Vegetative ''cuttings'' of original Villefranche (Z-series) polyp colonies propagated on glass slides are available on request to groups wishing to adopt the *Clytia* model. These strains were derived from a founder colony (Z) produced by adults and collected in the Villefranche-sur-mer Bay (France) and self-crossed for two or three generations. Self-crossing is possible in certain young colonies producing both male and female medusae in a temperature-dependent manner [40]. The colonies that we use routinely for experimentation (Z^4B (female), $(Z^4C)^2$ (male) and various crosses of these two strains) have fixed sexes.

Genome

Clytia is likely to be the first jellyfish genome to be sequenced and annotated [10]. Sequencing is underway at the Genoscope (Evry, France) using mainly 454 and Solexa technologies. Sequence completion is scheduled for April 2010 with public availability of an annotated sequence interface programmed for 2011. Genomic DNA for this project was obtained from male medusae from the $(Z^4C)^2$ colony obtained by three generations of sexual self-crossing. This genome is thus predicted to be homozygote for roughly seven out of eight genes, which should considerably aid sequence assembly. Furthermore, much of the genome sequence will correspond exactly to that of laboratory strains used routinely for experimental manipulation, greatly facilitating functional approaches.

Transcriptome

The current transcriptome collection of about 90,000 ESTs and 8000 full-length cDNA sequences corresponds to about one-third of the genes predicted in the fully sequenced *Nematostella* and *Hydra* genomes. Coverage will be extended by mass cDNA sequencing, including stage-specific RNA populations (programmed for 2010–2011).

Existing

(1) 81,976 5′ ESTs derived from 91,008 arrayed cDNA clones from two separate mixed stage (embryo, larva and medusa) libraries. NB 74,118 of these clones (EST prefixes SA) were from a library derived uniquely from Z-series colonies
(2) 6042 3′ ESTs from unique clones selected from the same starting libraries
(3) 2257 fully sequenced unique DNA clones selected from the same starting libraries.

These transcriptome data have been compiled to give 8053 unique protein-coding genes.

Clytia ESTs are freely available through NCBI dbEST (http://www.ncbi.nlm.nih.gov/dbEST/), and, along with Phrap assemblies, from the Compagen comparative genomics platform for early branching Metazoa (http://compagen.zoologie.uni-kiel.de/).

Upcoming

454 sequencing runs are programmed within the genome project for three cDNA populations:
(1) Adult transcriptome: $(Z^4C)^2$ male medusae plus $(Z^4C)^2 \times Z^4B$ polyps
(2) Maternal transcriptome: Z^4B female gonads and ovulated eggs
(3) Embryonic/larval transcriptome: mixed stages of embryonic and larval development $(Z^4C)^2 \times Z^4B$

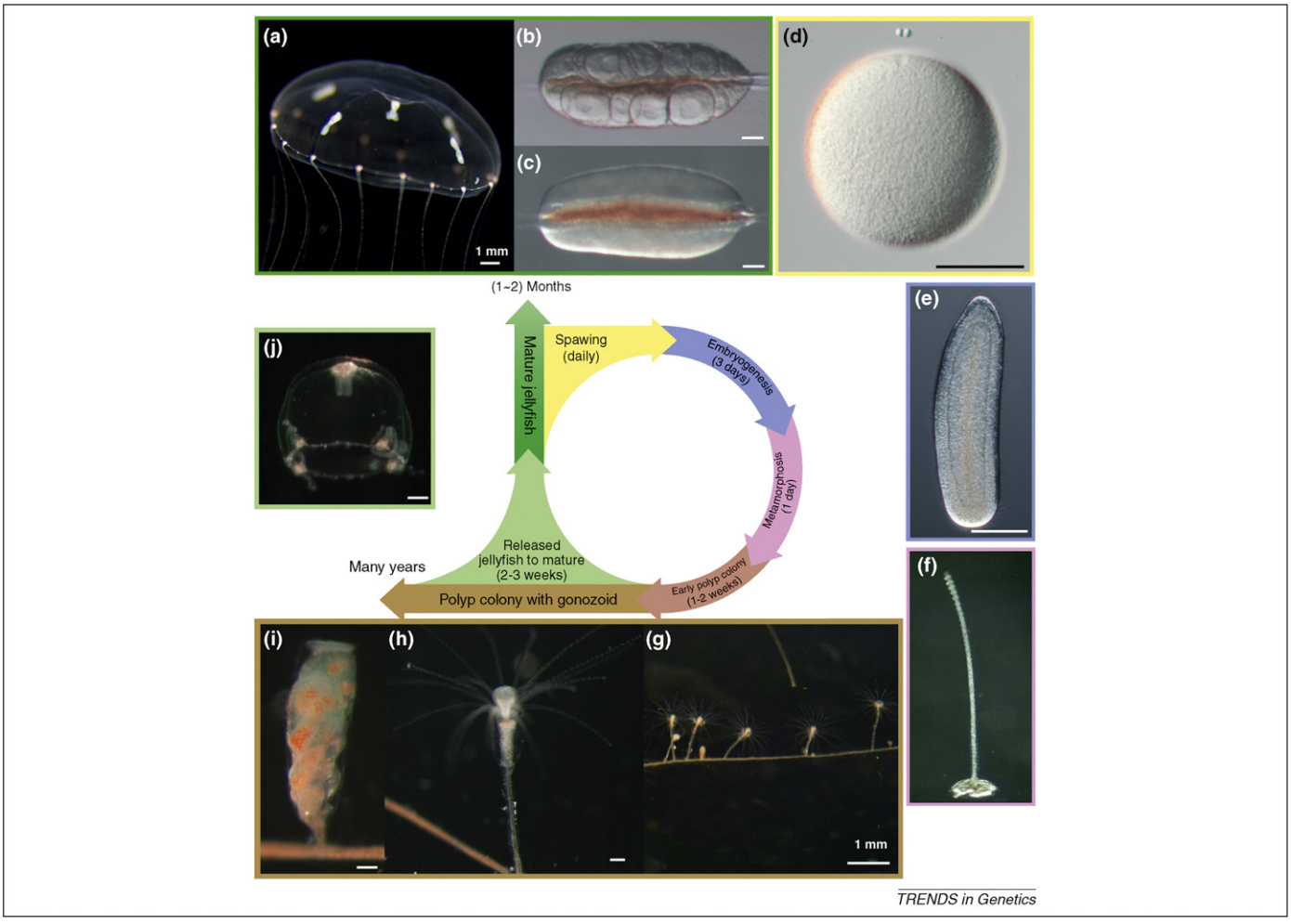

Figure 2. *Clytia hemisphaerica* life stages and culture. Adult medusae **(a)** are about 1 cm in diameter and can be cultured at a density of 10–12/litre of natural or artificial seawater. All polyp and medusa stages can feed on slightly aged artemia larvae as a convenient food source. Gonads of female **(b)** medusae are easily distinguishable from males **(c)** by the presence of growing oocytes of different stages. Ovulated eggs **(d)** are fertilized externally and a swimming planula larva forms after 1 day. At 3 days post fertilization the mature planula **(e)** can undergo metamorphosis **(f)**, experimentally inducible by treatment with CsCl [38]) to produce a primary polyp. Once it starts feeding, the primary polyp propagates vegetatively by stolon extension across the seabed (or glass beakers or slides) to form a connected colony **(g)** displaying two types of polyps: feeding gastrozoids **(h)** and reproductive gastrozoids **(i)**, from which baby medusae form by budding [82]. The colony lives eternally, with old parts being progressively replaced as the colony continues to spread. Newly released baby medusae **(j)** reach maturity after 2–3 weeks of feeding. The scale bars represent 1 mm for adult jellyfish and polyp colony, and 100 μm otherwise. Practical tips on Clytia culture are regularly updated at: http://biodev.obs-vlfr.fr/recherche/houliston/Clytia/Clytia.html.

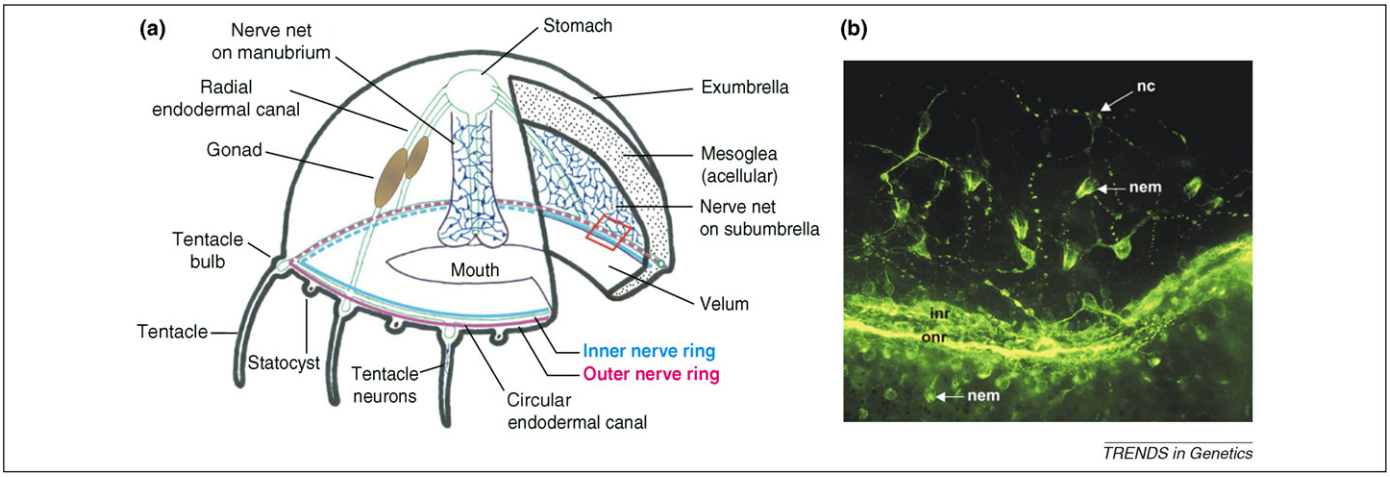

Figure 3. Organization of the *Clytia* nervous system. **(a)** Diagram of *Clytia* medusa anatomy, highlighting the main elements of its neurosensory system. The nervous system comprises diffuse epithelial nerve nets (on the subumbrella face, the manubrium and the tentacles) and two condensed nerve rings running along the periphery of the umbrella. Sensory organs are present in the form of simple statocysts (balance organs) located between tentacle insertion points. **(b)** Detailed view of the nervous system in the area corresponding to the red box in **a**, as revealed by anti-tyrosylated α-tubulin antibody staining. The two nerve rings (inr, inner; onr, outer) are visible, as well as part of the peripheral part of the subumbrellar nerve net, comprising nerve cells or ganglion cells (nc) and nematocytes (nem).

material for a key body of experimental embryology by Gary Freeman and collaborators [34–38].

A key advantage of *Clytia* as an experimental model is that the complete life cycle can be reproduced under controlled laboratory conditions (Figure 2) [39,40]. Male and female adult *Clytia* medusae spawn daily, triggered by light after a period of darkness [41]. Embryonic development (Figure 4a), proceeds through a series of cleavage divisions to produce a single cell-layered blastula, which forms cilia for swimming just before the onset of gastrulation. Gastrulation by unipolar cell ingression [42] produces a simple, two-layered planula larva with a polarized torpedo shape that swims directionally with the more rounded aboral end in front. The planula has no mouth, but upon metamorphosis the oral pole gives rise to the hypostome (mouth) of the primary feeding polyp. Once it starts feeding, the primary polyp propagates vegetatively by sending stolons out across the seabed or other substrate. The mature colony is a connected system of feeding gastrozoids (Figure 2 h) and gonozoids: polyps specialized for the budding of new baby medusae (Figure 2i). The colony can live for many years and can be considered immortal, old parts being progressively replaced as the colony continues to spread.

Clytia polyp colonies produce new medusae clonally, and so laboratory medusae produced from a single colony are genetically identical. Furthermore, male and female medusae with identical genotypes can be generated from a single colony by altering the temperature [40], enabling the generation of stably propagating colonies with a high degree of genetic homogeneity by self-crossing over several generations. Thus, all experiments in our laboratories on the cosmopolitan species *C. hemispherica* have been done for several years on a very small number of individuals with constant and highly uniform genotypes; notably, one related female and male pair (Z^4B and $(Z^4C)^2$). This is, of course, a big advantage for gene function analysis as well as genome sequencing (Box 1).

The combined practical advantages of *C. hemisphaerica* have allowed rapid development of reliable experimental approaches to test gene function during embryogenesis. Injection of morpholino (MO) antisense oligonucleotides into oocytes before fertilization can be used to achieve gene loss-of-function by translational inhibition, and injection of synthetic RNAs into eggs or single cells of the embryo to achieve gene gain-of-function [43–45]. The efficacy of the MO approach in *Clytia* is probably due, in large part, to the very low genetic polymorphism of laboratory reared medusae (see above). Polymorphism is a notorious source of problems in model species obtained from natural or heterogeneous populations. Furthermore, *Clytia* MO target sequences can be deduced exactly from a cDNA dataset derived from the same laboratory colonies as the jellyfish used for experimentation (Box 1).

Insights into development and evolution from *Clytia*

Published research on *Clytia* at the molecular level has come mainly from our two groups, and so reflects our

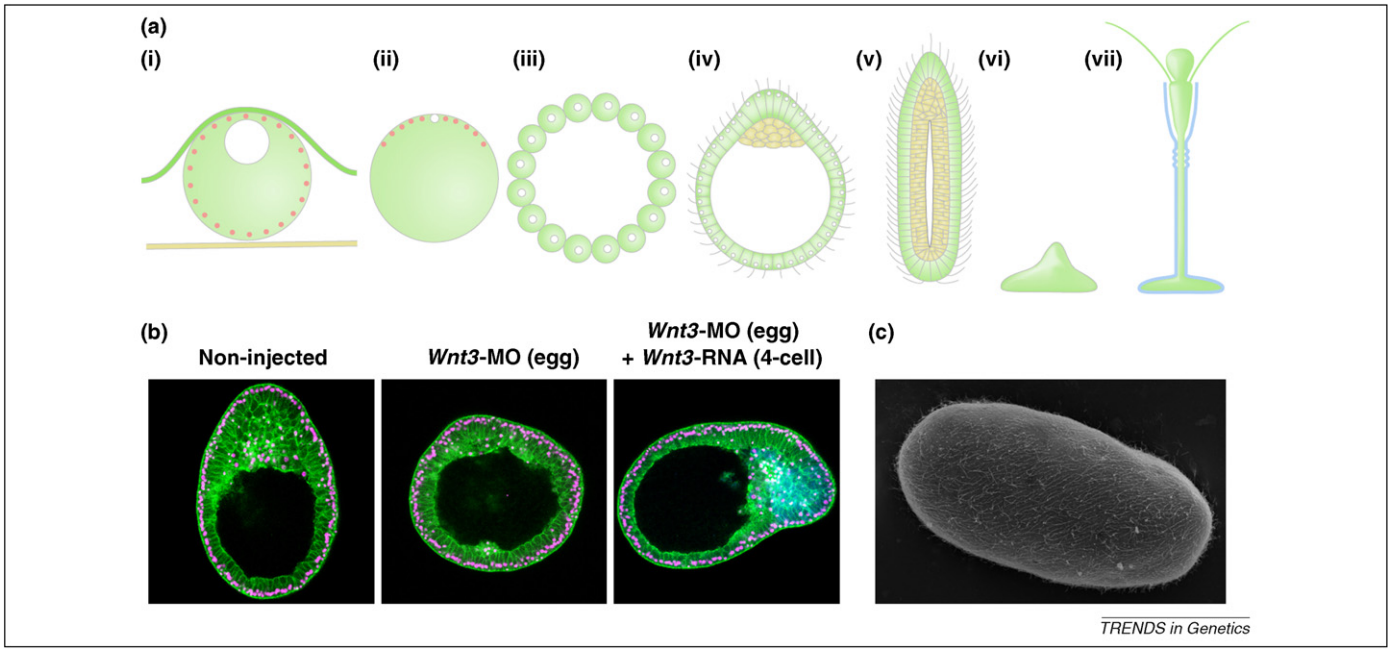

Figure 4. Development of polarity during embryonic and larval development. **(a)** A schematic representation of development from oocyte to primary polyp. The origin of larval polarity can be traced back to oocyte polarization during growth in the gonad (i) in which the oocyte nucleus becomes positioned apically with respect to endodermal (beige) and ectodermal (green) cell layers [52]. Oocyte polarity is reinforced during spawning and oocyte maturation (ii), when cortically localized mRNAs such as *Wnt3* (red dots) adopt asymmetric polarised localizations. Fertilization is followed by a series of unipolar, symmetric, synchronous cleavage divisions to produce an irregularly shaped, single cell-layered blastula (iii). The blastula cells then undergo an epithelialization and grow cilia, the embryo starting to swim just before the onset of gastrulation (about 10 h post fertilization at 18 °C). (iv) Gastrulation is initiated on the original animal side, and proceeds by unicellular ingression, usually from a single site as in *C. gregarium* [42] or occasionally from split sites to produce a two-layered planula larva (v). The gastrulation site becomes the oral pole of the planula and, upon metamorphosis (vi), the hypostome (mouth) of the primary gastrozoid (vii). **(b)** Confocal images of early gastrula stage embryos (green, phalloidin staining of cell boundaries; purple, TOPRO-3 stained nuclei), fixed when the oral pole (top) in uninjected embryos had already adopted its typical pointed morphology, and presumptive endoderm cells were ingressing from this site to fill the blastocoel. Injection of an MO to inhibit translation of maternal mRNA for the ligand Wnt3 prevents polarity development and gastrulation initiation [44]. *Wnt* mRNA injection into a single cell at the four-cell stage (descendants labelled blue) restores the oral pole in relation to the injection site, demonstrating that this ligand is both necessary and sufficient for embryonic axis specification. **(c)** Scanning electron microscope image of a *Clytia* planula. The polarity of the ectoderm is manifest by the alignment of the cilia, which cause directional swimming with the more rounded oral end in front.

particular scientific interests: the regulation of oogenesis and embryonic patterning, and the evolution of developmental mechanisms. We will illustrate the experimental possibilities offered by *Clytia* by describing briefly some of our first significant findings in these areas.

Embryonic polarity specification: conserved and derived features

The body plans of multicellular organisms can be defined in terms of symmetry properties and axes of polarity. Bilaterian species have two principal body axes (dorsoventral and anteroposterior), whereas most cnidarians, ctenophores and sponges (in larval stages at least) have a single main axis of polarity (oral–aboral). The molecular mechanisms that govern the formation of these axes, and the relationships between the polarities of bilaterian and non-bilaterian animals, remain fundamental unresolved issues in evolution and developmental biology [46]. In *Clytia,* formation of the oral–aboral axis has proved experimentally tractable and can thus help to illuminate this issue.

In *Clytia* as in other cnidarians, the oral pole of the larva derives from the animal pole of the egg. The orientation of larval polarity is, however, extremely sensitive to experimental manipulation of the egg and early embryo [34–36]. For this reason, polarity was long thought to emerge progressively during early cnidarian development rather than conforming to the general bilaterian principle of axis specification by maternal determinants. Recently, however, the picture of cnidarian polarity establishment has been brought back into line with that of bilaterians by studies on *Nematostella, Podocoryne, Hydractinia* and *Clytia*, providing direct or indirect evidence for the existence of maternal localized determinants that act via activation of the canonical Wnt signalling pathway [47–50,43,51,44]. In *Clytia,* these determinants have been identified at the molecular level as maternal mRNAs that become localized at the egg animal pole during oogenesis [43,44,52]. These RNAs code for Wnt ligands and receptors with antagonistic activities that cooperate to restrict Wnt pathway activation to the future oral territory. Thus, injection of specific inhibitory MOs into eggs before fertilization to prevent translation of these RNAs prevents the development of embryonic polarity almost completely, and introduction of synthetic RNAs into single blastomeres can restore polarity and reorient the axis of MO-injected embryos (Figure 4b). It is not yet known whether equivalent mRNAs act as determinants in other cnidarian species, in which various other potentially important localized mRNA and protein regulators of the Wnt pathway have been detected [48,51]. Whatever the diverse maternal cues that operate in different extant bilaterian and cnidarian species, it appears that Wnt signalling, probably involving both ligands and receptors, was of key ancestral importance for early embryonic patterning events in metazoans, evidence from sponges and protostomes now having been added to the cnidarian and deuterostome examples [53,54,46].

Following the initial definition of the oral and aboral poles via Wnt signalling during the cleavage blastula stages, axial patterning continues during gastrulation and larval development with the specification of distinct sets of cell types according to their position along the planula body axis [55]. Little is known about this process, although the nested and overlapping expression patterns of Wnt family genes, described in *Clytia* as in *Nematostella* and *Hydra,* might play a role [44,5,56,57]. In any case, it appears that the use of Hox and Parahox genes in defining positional identity along the body axis, well known for its conservation in bilaterian species [58], was an innovation of the bilaterian lineage. This is suggested by recent expression studies in *Clytia* [59,60] and other cnidarians [61] indicating the absence of conservation of a 'Hox code' in cnidarians, contrary to earlier indications [62]. Among *Clytia* Hox and ParaHox genes, only those belonging to the HOX9-14 and the Cdx groups exhibit restricted expression along the oral–aboral axis during development and in the planula larva; the others being expressed in very specialized areas at the medusa stage [59]. There is no conservation of the polarity of expression even between closely related Hox paralogues. Among three *Clytia* orthologues of the bilaterian posterior Hox genes (HOX9–14), two are expressed at the oral pole and one at the aboral pole of the planula larva. Of these, the closest *Clytia* orthologue of the sea anemone aboral gene *Anthox1* is expressed orally. Together with data from other hydrozoans [61], these observations reveal strong variability of Hox and ParaHox gene expression along the oral–aboral axis among cnidarian lineages, in contrast with the situation observed in bilaterians.

Lessons from Clytia stinging cells: nematogenesis and horizontal gene transfer

One of the unique defining characteristics of the Cnidaria is a remarkable cell type specialized for killing, the nematocyte or stinging cell. Each nematocyte contains a sophisticated pressurised capsule (the nematocyst) containing a rapid-firing, harpoon-like dart and lethal toxins [63]. Despite these extraordinary features, nematocytes can be considered to be a specialized neural cell type and exhibit many characteristics typical of neurosensory cells, including mechanosensitive cilia, neurite-like outgrowths and synapses, and the expression of conserved neural genes [23]. As nematocysts are single-use darts, nematogenesis (the generation of nematocytes) occurs continuously throughout larval and adult life. Nematogenesis provides one tractable non-bilaterian example of neurogenesis, and can provide insights into the genetic mechanisms underlying the origin of phenotypic novelties: unique features relating to stinging.

The molecular characterisation of nematogenesis comes mainly from work in *Hydra,* showing that nematocysts derive from a scattered population of stem cells (interstitial cells), which also generates other neural cell types, gland cells and germ cells [64]. In the *Clytia* medusa, the spatiotemporal characteristics of nematogenesis are particularly favourable for investigation because production of nematocysts to arm the tentacles is organised in an orderly manner in the tentacle bulb, a specialized swelling of the tentacle base (Figure 5) [65]. The tentacle bulb ectoderm is polarized, with a clear progression of successive nematoblast stages from the proximal bulb zone to its distal end where the tentacle starts. Interstitial cells

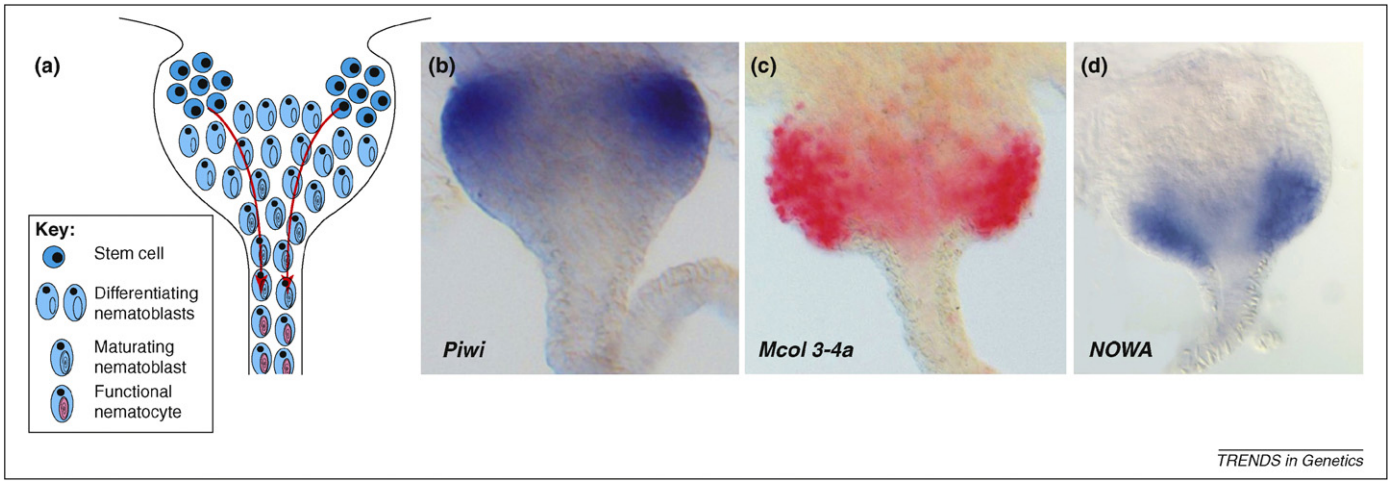

Figure 5. Nematogenesis in the medusa tentacle bulb. The tentacle bulb is a specialized swelling of the tentacle base (see Figures 2a and 3a). **(a)** Stem cells are concentrated in two groups at the base of the bulb. The successive stages of tentacle nematocyte differentiation (simplified from Ref. [65]) show a gradient distribution from base to tip, and they are displaced continuously towards the tentacle (shown by arrows). Genes involved in tentacle nematogenesis have crescent-shaped expression zones, the position along the bulb axis correlating with the time frame of gene function: **(b)** basal crescent for stem cell markers (e.g. *Piwi*); **(c)** median crescent for early differentiation genes (e.g. *mcol 3-4a*); **(d)** apical crescent for late differentiation/maturation genes (e.g. *NOWA*).

expressing stem cell markers such as *Piwi* [65] are positioned in the proximal region of the bulb, whereas other known nematogenesis-associated genes (*dickkopf-3, minicollagens* and *NOWA*) are expressed in compact domains staggered along the bulb ectoderm, corresponding to successive stages of nematogenesis (Figure 5b–d). The tentacle bulb system should thus be helpful for the systematic identification of genes involved in the various phases of nematogenesis from stem cells to functional stinging cells. Depending on successful development of RNAi and transgenesis approaches (see below), it also has potential for functional analysis of the regulatory genes implicated in stem cell maintenance and in nematoblast differentiation, because isolated bulbs have the remarkable capacity to continue to produce nematocytes and regenerate tentacles for several days when cultured in simple conditions.

Studies of *Clytia* nematogenesis have also provided an unexpected example of a particular genetic reorganization, lateral gene transfer, that might have contributed to the acquisition of phenotypic novelty in the phylum Cnidaria. An orthologue of the bacterial enzyme subunit *pgsA* (*capA*) is expressed in the nematogenic region of the tentacle bulb, where it is probably involved in the synthesis of polyglutamate, like its bacterial counterparts [66]. Polyglutamate is a very rare polymer known to occur only in cnidarians and in some bacteria. Its accumulation in the nematocyte capsules of cnidarians is essential for buildup of the pressure necessary for discharge [67]. Phylogenetic analysis suggested that *pgsA* jumped from a bacterium to the genome of a metazoan or cnidarian ancestor by lateral gene transfer [66]. Lateral gene transfer of *pgsA* might thus have had an important impact on nematocyte origin, and thus on the evolution of an entire animal phylum.

Identifying conserved mechanisms in gametogenesis

Clytia medusae possess four well-structured gonads, positioned midway along each endodermal radial canal (see Figures 2a and 3a). Gametogenesis is regulated in an orderly progression from a population of interstitial cells sandwiched between the endodermal and ectodermal layers [41]. Remarkably, gonads cut from adult female medusae can undergo repeated dark/light entrained cycles of oocyte growth and meiotic maturation for several days in culture [52]. The gonad is transparent, which offers a quasi-unique access to observe the successive stages of oocyte growth and maturation to microinjection. *Clytia* thus provides an excellent system for the assessment of gene function during oogenesis and oocyte maturation, which are key features of animal development [41]. To illustrate this, a functional study of the highly conserved meiotic regulatory kinase Mos using MO and RNA injection has revealed that this animal-specific kinase regulates meiotic spindle positioning and polar body emission in *Clytia* oocytes, and is required for post-meiotic cell cycle arrest. These roles feature amongst the various roles described for Mos kinases in classic bilaterian model species (notably, mouse, *Xenopus* and starfish), and are thus likely to represent the ancestral animal functions of this kinase [45]. By contrast, cell cycle progression is not affected by *Clytia* Mos manipulation, suggesting that any involvement in this process in bilaterians was acquired more recently. The *Clytia* study has thus helped to clarify our understanding of Mos function in oocyte maturation.

Perspectives for *Clytia* research

From the examples given above, it can be seen that *Clytia* can provide insights into embryonic pattering mechanisms, oogenesis, nematogenesis and stem cell biology. Given the remarkable regenerative abilities of both medusa and larval stages [26,35,38], and of isolated structures such as gonads and tentacle bulbs to function autonomously (see above), another promising avenue for *Clytia* research is the study of tissue homeostasis and regeneration. Furthermore, the presence of a medusa form gives *Clytia* the potential to help resolve a number of key issues in development and evolution pertaining to the acquisition of new phenotypic traits (Box 2).

Box 2. Jellyfish and the evolution of animal complexity

The presence of a complex medusa form in *Clytia*, equipped with sensory organs and striated muscle, should enable the study of a number of fundamental questions pertaining to the increase of animal complexity. These include:

- **The genetic basis of new traits**. What genomic changes underlie the evolution of a particular novel phenotype (in this case the medusa)? This issue is explored more thoroughly in Ref. [12] in this issue.
- **Epigenetic mechanisms governing animal form**. The polyp and medusa forms of *Clytia* provide a striking example of fundamentally distinct phenotypes encoded by the same genome. Study of their respective developmental programmes should help shed light on how such a situation can arise.
- **Converging mechanisms in nervous system evolution**. Bilaterian and medusa neurosensory systems show similar features, such as statocysts and functional specialization of nerve tracts, but phylogenetic studies indicate that these similarities reflect convergence following separate evolutionary origins. It will be instructive to investigate whether any deep homology of regulatory molecular mechanisms underlies the high degree of neurosensory regionalization of medusae and bilaterian central nervous systems.
- **The evolutionary origin of mesoderm**. Cnidarians have historically been considered to be diploblasts possessing only two germ layers (ectoderm and endoderm), with mesoderm hypothesized to have been an important evolutionary acquisition of the bilaterians (triploblasts). However, several lines of argument, notably concerning the expression of 'mesodermal marker' genes, have suggested that mesoderm might have had a more ancient origin during early animal evolution, with subsequent reduction or loss in some cnidarians [17,83,84]. Alternatively, these genes could have been involved ancestrally in regulating cell movement during gastrulation and/or in the specification of an endodermal territory from which bilaterian mesoderm was later derived [85–88]. This fascinating issue remains hotly debated [85,89–92].
- **The evolutionary origin of striated muscle**. Well-defined striated muscles are clearly present in cnidarian medusae but not obvious in polyps, raising the question of whether striated muscles originated separately in bilaterians and cnidarians, or have been lost in polyps from a striated-muscled ancestor. This issue is related to the mesoderm debate but is distinct, because muscles are not necessarily mesodermal in origin. Indeed, cnidarian muscles are typically formed by epitheliomuscular cells of the ectoderm or endoderm [85,61,89,90]. Furthermore, although striated muscle used for swimming in hydrozoan medusae develops from a mesodermal-like cell layer called the entocodon during medusa bud formation [17,83,84,90], this entocodon appears to be unique to hydrozoans [12].

New functional approaches

For the various biological processes listed above, expression profiling and phylogenetic analyses of *Clytia* orthologues of known bilaterian regulators should provide much useful information; however, analysis of gene function will require further technical developments. Currently, gene function analysis in *Clytia* is restricted to early developmental stages, as it relies on microinjection of MOs or synthetic RNAs (coding for wild type or mutant forms) into large oocytes, eggs or early embryos. An important future direction for the *Clytia* model is to develop experimental approaches allowing gene function analysis in the adult. Three avenues are being explored: RNAi, transgenesis and classical genetics.

RNAi has been used to reduce gene activity in *Hydra* polyps in regeneration studies [68–70]. Interfering double-stranded RNA constructs were administered by feeding with expressing bacteria over several days, resulting in measurable reductions of RNA and protein levels of the target genes. This approach has yet to be widely adopted, and may not be easily applicable to achieve rapid gene knockdown during specific events within the polyp. As well as testing the feeding approach in *Clytia*, it would be useful to try to introduce dsRNA directly into the endodermal cavities of isolated *Clytia* tentacle bulbs and gonads (see above) to address specific biological processes relating to nematogenesis, tentacle regeneration and oogenesis.

The use of transgenic lines to analyse gene regulation is being pioneered in both *Hydra* and *Nematostella* [71,72]. Studies with *Clytia* using the meganuclease technique [73] are underway, aimed initially at producing stably propagating transgenic polyp colonies to provide medusae expressing selected reporter genes, such as fluorescently tagged cytoskeletal, nuclear or cell type-specific markers. Depending on the success of RNAi approaches, it might be possible to use dsRNA-expressing transgenes for RNAi-mediated gene knockdown in polyp and medusa stages.

Clytia is potentially favourable for classic genetics (i.e. mutagenesis) screening for particular phenotypes and then identification of the mutated gene. The full cycle can be achieved in 6–8 weeks (Figure 2), and the polyps survive for many years, simplifying the maintenance of individual genotypes. The possibility of self-crossing and low genetic background facilitating mutant analysis should ease the production of homozygous genotypes. One possible avenue to mutagenesis in *Clytia* is transposon-mediated insertional mutagenesis and gene trapping, which are used successfully in another marine model animal, *Ciona intestinalis* [74].

Developing sequence resources

The existing transcriptome sequence collection has proved invaluable for the rapid identification, characterization and functional analysis of cnidarian orthologues of developmental regulatory genes [7,6,43,44,65,66,60,59,45]. It has also enabled *in silico* analyses addressing the phylogenetic relationships between non-bilaterian phyla [75], the evolution of mRNA processing by the addition of *trans*-spliced leaders [76,77] and the identification of potential medusa-specific genes [12]. Extension of *Clytia* transcriptome coverage is programmed within the genome sequencing and annotation project currently underway in collaboration with a team at Genoscope (Box 1). As well as being an invaluable resource for existing and future groups working on *Clytia*, the sequenced genome will enrich comparative genomic studies by allowing the definitive assessment of gene repertoires (e.g. diversification of multi-gene families) in a full life cycle cnidarian, and the comparison of gene regulatory elements including promoters, transposons and small regulatory RNAs.

Concluding remarks

C. hemisphaerica has rapidly established itself as a cnidarian experimental model capable of illuminating evolutionary and developmental questions. Specific advantages with respect to the existing cnidarian models include the existence of a complex medusa form, experimental accessibility of gametogenesis and larval development, and the availability of clonally propagating

laboratory colonies. The upcoming availability of the genome sequence along with the extension of functional approaches for genetic manipulation to adult stages should allow *Clytia* to become a valuable addition to the suite of laboratory models available for understanding developmental processes and early animal evolution.

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