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Cnidarians as a Model System for Understanding Evolution and Regeneration

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The Developmental Interest of Cnidarians

Cnidarians are simple animals that display either a simple tube-shape form, called the polyp, or a more sophisticated morphology named medusa. Cnidarians are made up of two multifunctional epithelial muscle layers separated by an extra-cellular substance named the mesoglea. Additionally the tissues contain nerve cells, nematocytes, interstitial cells and when appropriate, gametes. Cnidaria differentiate structures along their body axis that perform specific functions, like the head at the apical pole, responsible for the active feeding behavior, and in the medusa, monofunctional tissues like striated muscle or complex sense organs, like lens eyes or statocysts (Bouillon, 1994). The Cnidaria phylum, which together with the Ctenophora (comb jellies) and the Porifera (sponges) represent the only surviving diploblast species (Fig. 1), is supposed to predate the protostome / deuterostome divergence, representing thus a sister group to the bilaterian species. The cnidarian species distribute among four distinct classes, the Anthozoa, Hydrozoa, Scyphozoa and Cubozoa. From the molecular data accumulated during the past ten years, it appears that among the four cnidarian classes, the anthozoans (sea anemone, coral) arose first (Bridge *et al.*, 1992; Bridge *et al.*, 1995; Odorico and Miller, 1997; Schuchert, 1993). However, the cellular and molecular complexity of the medusa organisation (see below) remains enigmatic (Boero *et al.*, 1998).

More than 99% of the cnidarians are sea-water animals, and according to their class, live as polyps exclusively (all anthozoans, some hydrozoans like *Hydra* or *Hydractinia*) or alternatively differentiate both forms (many hydrozoans, all scyphozoans and cubozoans, see Fig. 2). The polyp can bud, either to reproduce asexually as in hydra, or to develop the parental form, the medusa that will complete the sexual cycle. In addition to budding and sexual reproduction, all polyp species and many medusa types can regenerate. Additionally when dissociated into small tissue fragments or single cells, reaggregation and regeneration occurs in all species and life stages, especially good results are observed with polyps. These later events prove that the developmental programs can be reactivated whatever the age of the animals. In general polyp forms are regarded as immortal whereas the medusa dies after liberation of gametes, with one exception, *Turritopsis*, where all animals transforms into polyps again (Piraino *et al.*, 1996).

For developmental purposes, different cnidarian species display complementary advantages: the hydrozoan freshwater hydra

Abbreviations used in this paper: bHLH, basic helix-loop-helix; BMP, bone morphogenetic protein; CREB, cAMP Response Element Binding Protein; HLH, helix-loop-helix; HMP, *Hydra* metalloproteinase; dsRNA, double stranded RNA; Mef, myocyte enhancer factor; PKC, protein kinase C; RGD, arginine, glycine, aspartic acid; FMRF, phenylalanin, methionine, arginine, phenylalanine; TPA, 12-O-Tetradecanoyl-phorbol 13-acetate.

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is probably the most well know cnidarian because of its regenerative possibilities. It is easily maintained in the laboratory where grafting, budding, regeneration and reaggregation are amenable to experimentation. In addition, a collection of different strains and mutants is available. However, its sexual cycle is most often seasonal and not adapted for extensive manipulations (Fig. 2, Left). In contrast, the light-inducible and short sexual development of the marine hydrozoan species, *Hydractinia* and *Podocoryne*, make them suitable for genetic purposes, e.g. the study of axis formation in early development. Additionally *Podocoryne* has a full life cycle including medusa development, the life stage when formation of monotypical tissues like the striated muscle or the subumbrellar plate, complex nerve systems and sense organs, can be observed (Fig. 2 Right). None of these structures differentiates in the polyp form. In anthozoans, the corals display an annual sexual cycle that cannot take place under laboratory conditions; this, however, is possible with sea anemones.

History and Contribution of the Cnidarian Model System over the Last 260 Years

Abraham Trembley, a gentleman from Geneva, incidentally discovered hydra regeneration at the time he was teaching the children of a Dutch prince in the Netherlands (Trembley, 1744; Lenhoff and Lenhoff, 1986; see Fig. 1 of article by Buscaglia and Duboule in this issue, pp. 6). His approach was very original, because he was the first scientist to perform systematic animal experimentation in order to understand and describe the process he had discovered. The fact that the original question was: is hydra a plant or an animal, might have helped him to apply strategies that were so far reserved to plants. Whereas experimental cnidarian research was not continued in Switzerland after Trembley, it became an important study subject at the turn between the XIXth and the XXth centuries, when most developmental biologists were fascinated by the regeneration potential of cnidarians. The problem of axially polarized regeneration was already recognized and studied. Morgan investigating head regeneration in *Tubularia* proposed in 1905 that "a gradient of material is regulating the hydranth (young polyp) forming material, which decreases from the apical towards the basal end" (Morgan, 1905). In 1907, Child concluded from his studies on *Tubularia*: "we may regard polarity as an axial difference in the character and energy of reactions resulting from part physiological relations" (Child, 1907). At the same time, Elena Browne by inducing the formation of extra-heads upon grafting small pieces of hydra tissue, discovered the phenomena of tissue induction (Browne, 1909), a discovery that took place 18 years before that of Speeman's on vertebrates.

More recently, **Pierre Tardent** (1927-1997; Fig. 3) started again experimental work with cnidarians in Switzerland, a task he pursued together with his wife Ruth all along his scientific life that covered more than 40 years (Tardent, 1952; Grassi *et al.*, 1995). In his almost 200 publications, Pierre Tardent documented an immensely broad interest in cell and developmental biology of cnidarians, investigating cellular dynamics, sex determination and regeneration in hydrozoans, in the first place hydra. His PhD thesis dealt with the distribution and the histology of interstitial cells (see below) in the freshwater polyp hydra and the marine polyp tubularia (Tardent, 1952; Tardent, 1954). In his postdoctoral years, he followed his interest in cnidarians at the Zoological Station of Naples where he stayed for 10 years. Already at Naples but more so in Zurich where he became professor in 1968, he pioneered the application of the techniques of cell biology to cnidarian research. He continued with his main and early interest for the interstitial cells, analysing their migration pattern (Smid and Tardent, 1984), their developmental potential (Rich and Tardent, 1969) and their role in sex determination (Littlefield, 1984). At many occasions his laboratory attempted to culture this cell type (Martin and Tardent, 1962), a goal not achieved today yet. His interest also included gametogenesis (Zihler, 1972), differentiation of germ cells (Tardent, 1985), distribution of nerve cells (Tardent and Weber, 1976; Epp and Tardent, 1978), cell migration (Tardent and Morgenthaler, 1966), cell-cell recognition phenomenons, in both hydra (Stidwill, 1981) and marine cnidarians (Tardent and Buhrer, 1982). Among derivatives of interstitial cells, he investigated the nematocytes, his "favourite cell" (Tardent, 1995), their histology, development and migration (Rich and Tardent, 1969; Weber *et al.*, 1978; Tardent and Holstein, 1982; Tardent *et al.*, 1985), the physical and biochemical aspects of nematocyst explosion (Holstein and Tardent, 1984; Weber *et al.*, 1987; Aerne *et al.*, 1991) and the regulation of their replacement (Zumstein and Tardent, 1971). At different times his laboratory did experiments on sex determination (Tardent, 1966; Tardent, 1968; Littlefield, 1984; Tardent, 1985; Grassi *et al.*, 1995) and the role of transdifferention in regeneration (Smid and Tardent, 1982). Furthermore his laboratory investigated formation of the mesoglea (Epp *et al.*, 1986) and examined how hydra reacts to light stimulus (Tardent and Frei, 1969; Borner and Tardent, 1971). His interest in marine hydrozoans included the histology, development and regeneration of the eye of the *Cladonema* medusa (Weber, 1981) and the development (Frey, 1968; Schmid and Tardent, 1969; Brändli, 1971; Schmid, 1972), regeneration (Schmid and Tardent, 1971; Schmid *et al.*, 1976) and transdifferentiation (Frey, 1968; Schmid, 1972) of medusae.

Pierre Tardent influenced cnidarian research in many ways. Firstly, his own students (Volker Schmid, Robert Stidwill) and postdocs (B. Marcum, Lynne Littlefield, Thomas Holstein) carried on with projects they had initiated in his laboratory. Secondly, the knowledge accumulated by the group of Pierre Tardent on cnidarians stimulated the formation of new cnidarian groups, especially that of Alfred Gierer who, in the early 70s, decided to use hydra as a model system for understanding pattern formation

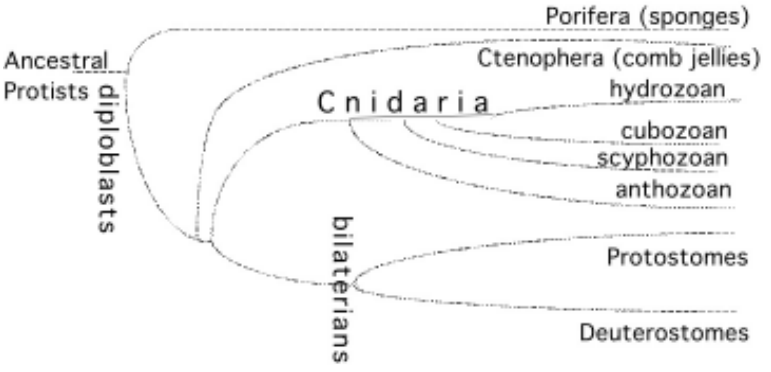

Fig. 1. Four distinct classes form the Cnidaria phylum. *Both* Hydra *and* Podocoryne carnea *belong to the hydrozoan class.*

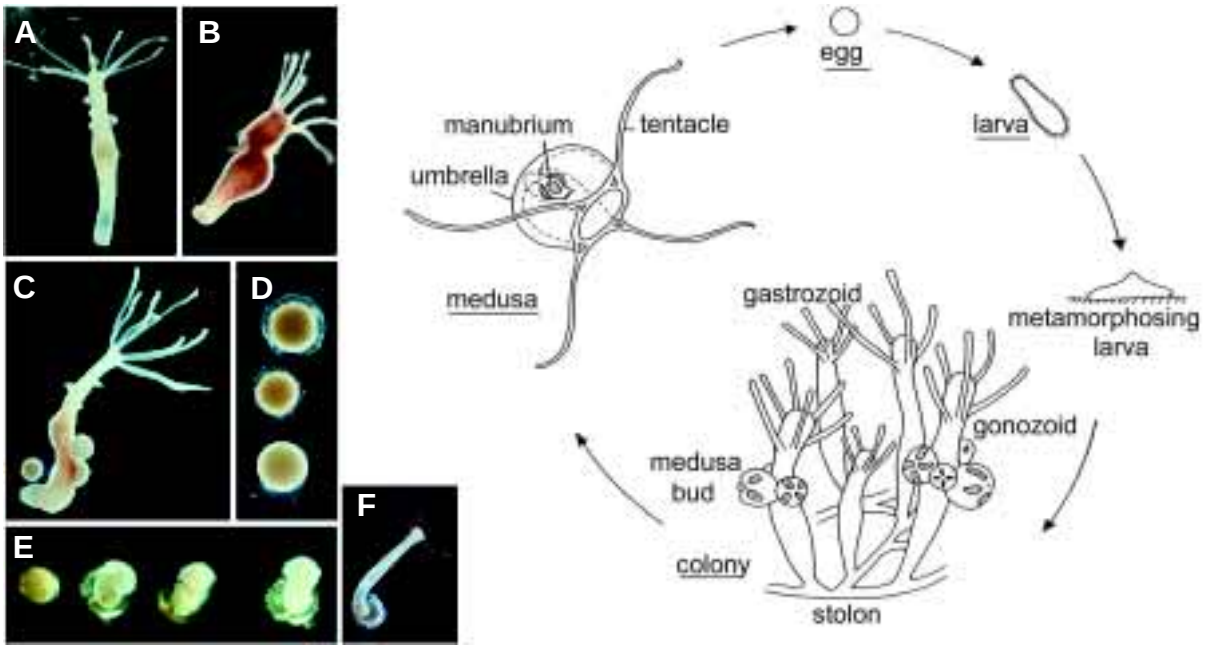

Fig. 2. Life cycle patterns in the Hydroidea (Hydrozoa, Cnidaria). (Left) *Sexual cycle of the freshwater hydrozoan,* Hydra vulgaris, *a strain obtained from Pierre Tardent which constitutively produces gametes (Grassi* et al.*, 1995). Testis are visible in* **(A,C)**, *ovary in* **(B)**, *fertilized eggs still attached to the parental polyp in C, detached embryos in* **(D)**. *After a variable period from 2 weeks to several months, a young hydra will hatch* **(E,F)**. **(Right)** *The life cycle of the marine hydrozoan* Podocoryne carnea.

processes. Together with his collaborators in Tübingen (Germany), Gierer discovered that cells obtained from dissociated hydra could reaggregate and reform a complete normal animal within a relatively short period of time (Gierer *et al.*, 1972).

Finally, as a contribution of the cnidarian model system to our current view of developmental mechanisms, hydra was the first species where conservation over evolution of molecules regulating cell differentiation and developmental processes could be demonstrated. Early investigations in the late 50s already proved the presence of substances which, once extracted from hydra and applied on regenerating animals, would promote the regeneration of head structures (Burnett, 1965). In the 70s, similar properties were demonstrated for the neuropeptide Head Activator (Schaller, 1973; Schaller *et al.*, 1979; Schaller *et al.*, 1989). This peptide was biochemically purified and sequenced from both sea anemone (Schaller and Bodenmüller, 1981) and bovine (Bodenmuller and Schaller, 1981) showing a strikingly identical sequence that proved a perfect conservation from cnidarians to vertebrates.

Genetic Conservation from Cnidarians to Bilaterians

The recent years were an active period for cloning evolutionarily conserved genes from cnidarian species. It is now clear that those species make use of highly evolutionarily-conserved genes that encode functional domains, involved in gene regulation, translational control, signal transduction, apoptosis, extra-cellular signalling, specification of the myogenic cell lineage and cell/extracellular matrix (ECM) interactions (reviewed in (Galliot, 2000). Furthermore migration of nematocytes seem to be controlled through RGD dependent ligand-receptor complexes (Ziegler and Stidwill, 1992) and the entire Wnt/wingless signalling pathway is conserved in hydra (Hobmayer *et al.*, 2000). These data prove first, that most if not all of the gene families do have representatives in cnidarians, and second, that their diversification in many cases occurred prior to the Cnidaria divergence.

Moreover, the conservation from cnidarians to chordates is remarkable. For example, the sequence of the mesoderm specification factor Twist in the bHLH domain is significantly closer to vertebrate than drosophila or nematode twist cognate sequences (Spring *et al.*, 2000). Cnidarians have actually retained gene families, like the Syk protein-tyrosine kinase (Steele *et al.*, 1999), the Not, Hex (Gauchat *et al.*, 2000) and Pax-3/7 (Miller *et al.*, 2000; Gröger *et al.*, 2000) homeobox genes, that have been lost in some bilaterian species, because they were not found in the nematode and/or Drosophila genomes. In addition, genomic data show the conservation of introns at fixed positions within functional domains (Galliot *et al.*, 1995). All together, this high level of gene conservation strengthens the validity of the cnidarian model systems for studying basic developmental processes shared by eumetazoans. Despite the lack of genetic tools currently available in cnidarian species so far, functional assays such as antisense (Yan *et al.*, 2000a) or dsRNA interference (Lohmann *et al.*, 1999) should rapidly highlight the developmental functions of these genes.

Apical and Axial Patterning in Cnidarians

Since the discovery of the conservation of the homeobox motif between Drosophila and vertebrates genes (McGinnis *et al.*, 1984; Scott and Weiner, 1984; Carrasco *et al.*, 1984), it became more and more obvious that pieces of evolutionarily-conserved developmental pathways were recruited for similar developmental tasks by protostomes and deuterostomes (Duboule and Wilkins, 1998). Such functional conservation implies that these developmental pathways were already present in their common ancestor. Thus, the cnidarians are the best candidates for investigating a non-bilaterian representation of these ancestral developmental path-

Fig. 3. Pierre Tardent, *at a conference (ca. 1970).*

ways. Those are supposed to trigger basic developmental functions, as neurogenesis, head patterning and positional information along the axis, that make possible an active feeding behavior and an active locomotion.

Recently, expression analyses performed at the quantitative and qualitative levels were made available from different cnidarian species (*Hydra*, *Podocoryne*, *Hydractinia*, coral) and permitted to distinguish genes that might exert a developmental function. Several of these evolutionarily conserved genes clearly show an expression that is regulated in time and in place where developmental events take place (Fig. 4). These molecular approaches have already brought some light on the conservation or the divergence of molecular mechanisms that support similar developmental events in cnidarians and bilaterians. For example, apical patterning in hydrozoans on one hand and anterior patterning in chordates on the other, use structurally-related genes like *forkhead* (Martinez *et al.*, 1997), *emx* (Mokady *et al.*, 1998), *paired-like* (Gauchat *et al.*, 1998; Broun *et al.*, 1999) genes. This suggests some conservation of ancestral basic mechanisms involved in head organizer activity (Galliot and Miller, 2000). However, axis formation from a pool of cells that will differentiate both an apical and a basal pole, as it occurs during budding, regeneration and reaggregation, also likely requires the activity of *Hox*-related (Schummer *et al.*, 1992; Gauchat *et al.*, 2000) as well as that of the *brachyury* (Technau and Bode, 1999) and the *Wnt*-pathway (Hobmayer *et al.*, 2000; Technau *et al.*, 2000) genes. Consequently, the apico-basal axis of the cnidarian polyp cannot be considered as a primitive bilaterian anterior-posterior or dorso-ventral axis (Gauchat *et al.*, 2000; Galliot, 2000). Axis formation is nevertheless a difficult question in cnidarians. As described above, the axis can be established in three different contexts in cnidarians: i) during sexual development, from the egg to the swimming planula (the larva that will metamorphoses into the polyp), ii) in the polyp at the time it buds or regenerates a new polyp (asexual reproduction), or iii) in the medusa at the time it buds from the polyp. Moreover, the larva and the polyp axis are often considered as inverted: the anterior larval pole will adhere to the substrate while the posterior larval pole will become the polyp mouth with tentacles. Are there common molecular mechanisms underlying these different processes? Which of these axis-forming contexts reflect at best the mechanisms at work in the ancestor common to cnidarians and bilaterians? Extensive comparative data are needed before we can answer to these questions.

Molecular Markers of the *Podocoryne carnea* Life Cycle

Although all essential elements and the diversity of the cnidarian life cycles are well established (reviewed in Bouillon, 1994), recent experimental and molecular data add new aspects in how the different life cycle stages can be viewed. The developmental

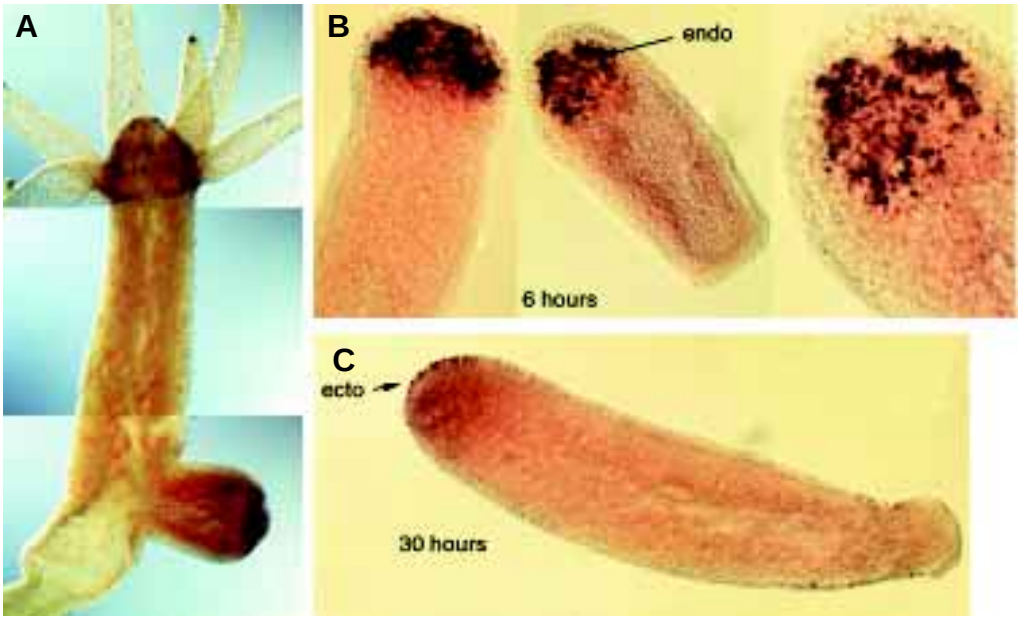

Fig. 4. The regulation of the homeobox paired-like gene *prdl-a* possibly reflects an ancestral function in apical patterning which was conserved and recruited for anterior patterning in bilaterians (Gauchat *et al.*, 1998; Galliot and Miller, 2000). (A) *In adult polyps,* prdl-a *displays an expression which is restricted to the nerve cell lineage of the head.* **(B,C)** *During regeneration,* prdl-a *shows a sequential expression, first in endodermal cells of head-regenerating stumps 6 hours after bisection (B) and later in the ectodermal cells of the regenerating head (C).* Prdl-a *shows a similar biphasic mode of expression during budding. In (A) expression of the* prdl-b *gene was simultaneously detected along the body column with a fluorescein-labelled probe (red dots). ecto, ectoderm; endo, endoderm.*

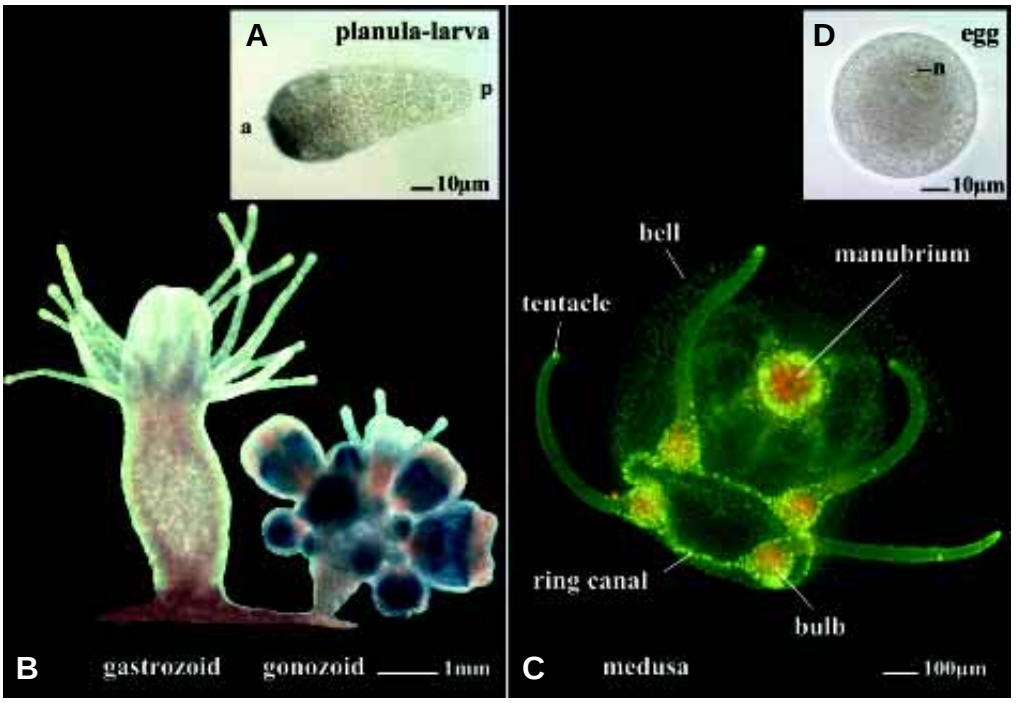

Fig. 5. Life cycle stages of *Podocoryne carnea*. (A) *Planula larva, the animal shows the graded anterior(a)-posterior(p) expression pattern of Cnox1-Pc, an anterior* Hox*-like gene.* **(B)** *Cnox1-Pc expression in the gastrozooid and the gonozooid. Staining is preferentially strong in the developing striated muscle tissue of the medusa buds.* **(C)** *Medusa stained with BrdU antibody which detects all nuclei, that have undergone DNA replication during the incubation time. Growth zones are located in the manubrium and at the periphery of the bell.* **(D)** Podocoryne carnea *egg. Nucleus and nucleolus(n) are located in the animal half.*

program of the full hydrozoan life cycle has two distinctive and separate stages: first, the formation of the planula larva and its transformation to the polyp; second, the formation of the medusa from polyp tissues (Fig. 5). It appears that all cell types and the bilayered body structure of the polyp have already differentiated in the young *Podocoryne* larva 25-36 hrs postfertilization (Gröger and Schmid, 2001). Whereas the differentiating smooth muscles, RFamide positive nerve cells and most nematocytes express no axial polarity, the tyrosine-tubulin positive nervous system develops gradually in repetitive patterns from anterior to posterior. In contrary, the large tyrosin-tubulin positive nematoctes are found only at the posterior end of the larva. This is paralleled, spatially and temporally, by the anterior expression of the *Podocoryne* genes *Cnox2-Pc* (an orphan Hox) in the ectoderm (Masuda-Nakagawa *et al*., 2000), and *Gsx (paraHox)* in the endoderm (Yanze *et al*., 2001). The posterior pole of the larva seems to be defined by *Cnox4-Pc*, an other orphan Hox gene, which is expressed as a maternal message in the egg at the site where first cleavage is initiated. At subsequent stages, *Cnox4-Pc* transcripts are exclusively detected in the few blastomere cells that localize to the future posterior pole (Yanze *et al*., 2001), the site of gastrulation (Freeman, 1981). Formation of the polyp occurs by settlement of the swimming larva and rearrangment of the cell types and tissues (Tardent, 1978). This process is not investigated yet at the molecular level.

In contrast to polyp formation, the development of the medusa is a *de novo* developmental process as the medusa differs in body structure and cell types from that of the polyp. The early medusa are formed from dedifferentiating polyp cells which proliferate intensively (Brändli, 1971; Bölsterli, 1977; Spring *et al*., 2000). Later in medusa development a third tissue layer separates from the ectoderm (Kühn, 1910; Bölsterli, 1977) forming the medusa bell that contains several non-myoepithelial cell types and sense organs (Tardent, 1978). The development of the third layer, called entocodon, and the fact that it cavitates and produces the smooth and striated muscle of the medusa bell has put forward the idea that hydrozoans are ancestral and derived triploblasts (Boero *et al*., 1998). This idea is supported by molecular data, which suggest that the structural genes (myosin heavy chain (Schuchert *et al*., 1993) or tropomyosin (Gröger *et al*., 1999)), the genes that specify the myogenic linage (*Twist* (Spring *et al*., 2000), *Brachyury*, *Snail* and *Mef* (Spring *et al.,* submitted)) and those regulating the muscle differentiation (the HLH family genes *JellyD* or *Id* (Müller *et al.,* in preparation)) are structurally and functionally conserved in *Podocoryne*.

The Hydra Regenerating Stump: a Paradigm for Organizer Activity

In *Hydra*, grafting experiments have provided the first hints about the regions where organizer activity distributes along the axis (Browne, 1909; Webster, 1966; Wolpert *et al*., 1971) and when this organizer activity appears in the regenerating stump (Berking, 1979; MacWilliams, 1983a; MacWilliams, 1983b). Two pairs of gradients, one each for the head and the foot, display activation and inhibition parallel graded activities along the body axis with the maxima in the head region for the head activation/ head inhibition, and in opposite directions for the foot activation/ foot inhibition. After amputation, a rapid and long-lasting drop of head inhibition was immediately observed, consistently with its major source of production in the head (MacWilliams, 1983a), while a delayed head activation (nowadays named head organizer activity) was progressively appearing in the tip of the regenerating-stump, reaching a plateau level about 10 hours after mid-gastric section (MacWilliams, 1983b) (Fig. 6A). At the cellular level, the restoration of lost structures occurs initially without cell proliferation but rather, by the direct differentiation of the stem cells and precursor cells present in the body column (Park *et al*., 1970; Holstein *et al*., 1991). Thus,

unlike axolotls, planarians or medusa, *Hydra* "bypasses" blastema formation and goes directly from wound healing to regeneration proper, a process named morphallaxis.

After *Hydra* bisection, gene regulation is detectable immediately (within 1 hour), early (within 10 hours), early-late (from 15 to 36 hours) or late (after 40 hours) (Fig. 6B). These "immediate", "early", "early-late" and "late" successive waves of gene regulation are concomitant with the wound healing phase, the establishment of organizer activity and the differentiation of head structures, respectively. In most cases, the "immediate" and very "early" gene modulations are observed in both head- and foot-regeneration stumps, implying that some components are shared at this stage. Recent work has demonstrated that most of the early regulatory genes can be detected in the endodermal cells of the regenerating stump (Galliot, 2000). In addition, when documented the expression of these "early" genes like *brachyury* (Technau and Bode, 1999) or *wnt* (Hobmayer *et al.*, 2000) are altered in the regeneration-deficient mutant *reg-16.* Taken together, these observations suggest that endodermal cells of the stump establish organizer activity in *Hydra* thanks to these "immediate/early" regulatory genes. At the "early-late" and "late" stages, genes are most often expressed as broad domains in the layer, either ectodermal or endodermal, that corresponds to the future adult expression domain. During the third day of head regeneration, these expression domains get restricted to reach the adult pattern.

How amputation activates or represses the expression of these developmentally regulated genes, however, remains a mystery. Amputation induces the release of "messenger" molecules, which lead to immediate modifications in DNA-binding activity (Galliot *et al.*, 1995) and transiently target regeneration-specific pathways (Hampe *et al.*, 1999), among which the PKC (Hassel *et al.*, 1998) and the CREB pathways (Kaloulis *et al.,* submitted). Such regulation may be mediated by peptides (Schaller *et al.*, 1996; Grens *et al.*, 1999; Hampe *et al.*, 1999; Lohmann and Bosch, 2000), low-molecular weight substances like fatty acids and their derivatives (HETEs) (Hassel *et al.*, 1996), ubiquitously distributed factors like hydroperoxides (Jantzen *et al.*, 1998), evolutionarily-conserved signals (Hobmayer *et al.*, 2000), specific metalloproteinases whose down-regulation prevents either head or foot regeneration (Leontovich *et al.*, 2000; Yan *et al.*, 2000a; Yan *et al.*, 2000b). These substances likely translate the physical stimulus of amputation into cascades of gene expression. The first stage in regeneration after amputation is the closure of the wound by cell and tissue migration.

The comparison of the expression patterns observed in the adult polyps with those detected in budding, regenerating or reaggregating animals implies that patterning is actively maintained in the adult animal by genetic networks that are extensively reprogrammed when the animal starts forming a new head, a new foot or a new axis. At the initial phase of budding, a specific genetic program that involves the *Otx* (Smith *et al.*, 1999) and the *wnt / b-catenin* (Hobmayer *et al.*, 2000) genes, takes place at the position along the body column where the bud will emerge. At the subsequent stages, genetic regulations are highly similar to that observed during regeneration of the head and the foot. Thus, except the initiation phase, budding and regeneration appear as the two faces of the same coin.

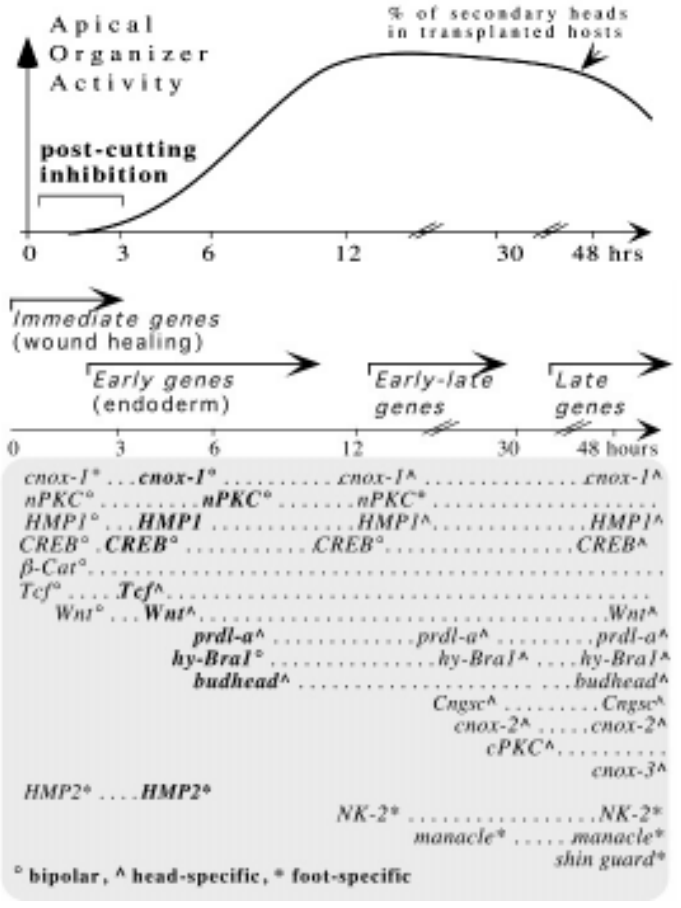

Fig. 6. Evolutionarily-conserved genes show early modulations in gene expression during hydra regeneration. (Upper panel) *The head organizer activity during regeneration was assessed by transplantation experiments: the head-regenerating stumps were grafted onto intact hosts at different times after amputation and the appearance of a secondary head was monitored 2 days later (Mac Williams, 1983b).* **(Lower panel)** *Induction of gene expression can be observed at different time points after mid-gastric section, either immediately (immediate genes), or within the first six hours (early genes), or after one day (early-late genes). Finally some head-specific genes start to be re-expressed when tentacle rudiments have appeared. Immediate or early genes can show successive distinct waves of expression. References:* budhead *(Martinez* et al.*, 1997); β-Cat, Tcf, Wnt (Hobmayer et al., 2000); Cnsgc (Broun et al., 1999); cnox-1, cnox-2, cnox-3 (Gauchat et al., 2000); CREB (Kaloulis, BG unpublished); HMP1 (Yan et al., 2000b);* HMP2 *(Yan* et al.*, 2000a); hyBra1 (Technau and Bode, 1999); NK2 (Grens et al., 1996); cPKC (Hassel, 1998); nPKC (Hassel et al., 1998); Manacle, Shin guard (Bridge et al., 2000).*

Instability of the Differentiated State

One of the central issue addressed by regeneration is that of the mechanisms that maintain the developmental program accessible in those species whatever the age of the animal. In principal the regenerate can be formed either by morphallactic rearrangement of the remaining tissues (sponges, some cnidaria), or by recruitment of undifferentiated cells (neoblasts in planaria), or by activation of differentiated cells through dedifferentiation and reprogram-

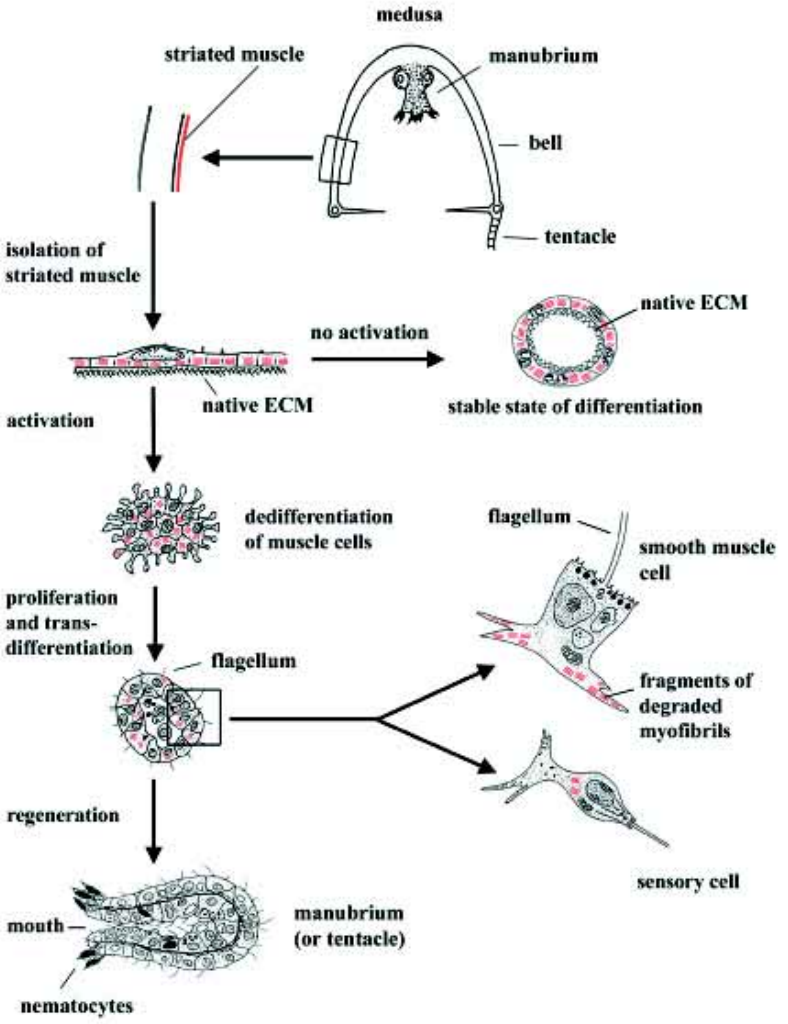

Fig. 7. Experimental conditions leading to transdifferentiation of striated muscle to smooth muscle and sensory cells in *Podocoryne carnea*.

ming of their cellular committment, a process called transdifferentiation. In general, cellular commitment and the differentiated state are stably controlled and in many cell types seem to be irreversibly fixed. Although transdifferentiation was mainly investigated in the context of regeneration (reviewed in Okada, 1991), more recent reports demonstrate that it can be also part of normal development (Bölsterli, 1977; Bode *et al.*, 1986; Schmid, 1992), even in vertebrates (Mochii *et al.*, 1998; Patapoutian *et al.*, 1995).

Cnidarians seem to be well suited to analyse the mechanisms of transdifferentiation. They regenerate well, consist of a small number of cell types and the mayor families of regulatory genes have less members than in higher phyla. Because it can be easily isolated as a monotypical tissue and cultured, the isolated striated muscle of medusae was intensively investigated for its transdifferentiation potential (reviewed in Schmid, 1992). When isolated by microsurgery together with portions of adhering ECM and cultured in artificial seawater without further treatment, the differentiated state of the striated muscle is maintained until the isolated muscle fragments disintegrate after 3-4 weeks. When activated for transdifferentiation, the striated muscle cells start to dedifferentiate and DNA replication is induced after 24 to 48 hours (Fig. 7). In all activated isolates flagella are formed *de novo* and smooth muscle cells and FMRFamide positive nerve cells develop. Occasionally striated muscle isolates are able to regenerate even the feeding and sexual organ (manubrium) and tentacles. In this case the striated muscle cells are able to transdifferentiate into smooth muscle cells, nerve cells, nematocytes. Whereas formation of smooth muscle cells needs no DNA replication, transdifferentiation to nerve cells requires one cell cycle, and all the other cell types two (Alder and Schmid, 1987).

Transdifferentiation of isolated striated muscle can be induced by treating this tissue with ECM degrading enzymes, by drugs activating the PKC such as TPA, mezerein or diacylglycerol, or by grafting muscle isolates onto pieces of ECM (reviewed in Schmid and Reber-Muller, 1995). Induction was also obtained with a mAb specific for ECM-specific carbohydrate moieties (Reber-Muller *et al.*, 1995). Taken together these results demonstrate that the stability of the differentiated state of striated muscle is controlled by the cell-substrate complex and thus confirms *in vivo* observations (Schmid *et al.*, 1999).

Since regeneration starts with wound closure that requires cell and tissue migration, the effect of change in cell substrate adhesion on gene expression was investigated by grafting isolated striated muscle on stretched ECM. The cells quickly adhere on the host ECM and migrate onto it until the muscle tissue is completly stretched (12-24 hrs). Surprisingly expression of striated muscle specific regulatory genes like the homeobox genes *Otx* and *Cnox1-Pc* (*Hox1*-like) and of the structural genes *MHC* (myosin heavy chain) and tropomyosin (*Tpm2*) are downregulated in the migrating cells whereas expression of the ubiquitously expressed homeobox gene *Cnox3-Pc* (*Msx*-like) is maintained (Yanze *et al.*, 1999). The fact that dedifferentiation is not initiated during migration indicates that additional activating pathways, possibly those stimulating and controlling cell cycle activity, are needed for the reprogramming of the genome and for the fixation of the newly determined state.

The investigations on gene regulation in the transdifferentiation process demonstrated that the genes specific for the striated muscle (see above) are turned off within hours postactivation (*Cnox1-Pc*) or 1-3 days (*Otx*, unpublished), others like *Pax-B* are permanently (Gröger *et al.*, 2000) or transiently, like *BMP2-8* (Reber-Müller, in preparation) turned on. Interestingly *Twist* which is required to make striated and smooth muscle in medusa development, is not expressed throughout the transdifferentiation process (Spring *et al.*, 2000), indicating that regeneration not necessarily copies ontogeny.

Conclusion

The question of the conservation of the molecular cascades that underly regeneration in different phyla, either invertebrate or vertebrate, remains open (Sánchez Alvarado, 2000). Identification of molecular markers at work during *Hydra* regeneration and *Podocoryne* transdifferentiation will allow the characterization in a close future of the key signaling cascades involved in cnidarian

regeneration. Present work suggests that most of these components are evolutionarily conserved, then deciphering their regulation could open the way to understand how a developmental program can be reactivated, especially in species where this program is locked very rapidly during development.

Summary

Hydra and *Podocoryne* are two cnidarian animals which provide complementary advantages for analysing developmental mechanisms possibly reflecting the basic developmental processes shared by most bilaterians. Interestingly, these mechanisms remain accessible all along the life of these animals, which bud and regenerate, whatever their age. The *Hydra* polyp permits a direct study of the molecular cascades linking amputation to regeneration. *Podocoryne* displays a complete life cycle, polyp and medusa stages with a fast and inducible sexual cycle and an unparalleled *in vitro* transdifferentiation potential. In both cases, a large number of evolutionarily conserved molecular markers are available, and analysis of their regulation highlights the molecular mechanisms which underly pattern formation in these two species.

KEY WORDS: *cnidarians, hydra, podocoryne, regeneration, budding, transdifferentiation, evolution, apical/head patterning, axial patterning, extra-cellular matrix, striated muscle*

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