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Hydra, a fruitful model system for 270 years

BRIGITTE GALLIOT*

Department of Genetics and Evolution, University of Geneva, Switzerland

ABSTRACT The discovery of *Hydra* regeneration by Abraham Trembley in 1744 promoted much scientific curiosity thanks to his clever design of experimental strategies away from the natural environment. Since then, this little freshwater cnidarian polyp flourished as a potent and fruitful model system. Here, we review some general biological questions that benefitted from *Hydra* research, such as the nature of embryogenesis, neurogenesis, induction by organizers, sex reversal, symbiosis, aging, feeding behavior, light regulation, multipotency of somatic stem cells, temperature-induced cell death, neuronal transdifferentiation, to cite only a few. To understand how phenotypes arise, theoricists also chose *Hydra* to model patterning and morphogenetic events, providing helpful concepts such as reaction-diffusion, positional information, and autocatalysis combined with lateral inhibition. Indeed, throughout these past 270 years, scientists used transplantation and grafting experiments, together with tissue, cell and molecular labelings, as well as biochemical procedures, in order to establish the solid foundations of cell and developmental biology. Nowadays, thanks to transgenic, genomic and proteomic tools, *Hydra* remains a promising model for these fields, but also for addressing novel questions such as evolutionary mechanisms, maintenance of dynamic homeostasis, regulation of stemness, functions of autophagy, cell death, stress response, innate immunity, bioactive compounds in ecosystems, ecotoxicant sensing and science communication.

KEY WORDS: *historical perspective, transplantation, modeling, developmental reactivation, Hydra regeneration, multipotency, stemness, symbiosis, environment*

The heuristic value of the *Hydra* model system

In the early 18th century the word biology was not yet in use but the nature of living organisms and their evolutionary relationships was the focus of interest for philosophers and naturalists, as evidenced by their pioneering efforts to develop new tools such as microscopes that would allow finer observation (Palm, 1996). The microscopic observation of organisms taken in the field undoubtedly helped develop morphological keys to sort between the animal and vegetal kingdoms and to group them into phyla (Linnaeus, 1758). Among the ambiguous species that could not be easily classified were the seawater corals that looked like flowers (Watson, 1753; McConnell, 1990) and the freshwater *Hydra* polyp that was considered to exhibit both animal and vegetal features. For example, *Hydra* easily reproduces asexually through budding, a trait frequently assigned to plants or fungi.

Having observed some *Hydra* polyps in a pond, Abraham Trembley (1710-1784) (who had received a PhD in mathematics from the University of Geneva (Switzerland) and was now educating the children of the Count of Bentick in the Netherlands) decided to solve that problem by testing their capacity to regenerate, assuming that if *Hydra* regenerates, then it should be considered a plant, but if it does not, then it should belong to the animal kingdom (Trembley, 1744). Thus, he cut this little organism into two pieces and observed during the next days that the *Hydra* had regenerated any cut away part. However, Trembley did not conclude that it was a plant as he had carefully noted several behaviors that were not consistent with it being a plant. Indeed he noted that *Hydra* actively capture their food with their tentacles, contract upon touch, slowly but efficiently walk (Trembley, 1744). All these behaviors that indicate the presence of nervous and digestive systems, are clear hallmarks of animals traits.

Abraham Trembley understood that that he had made a major discovery, i.e. an animal is able to fully regenerate any missing part of its body, and thereafter spent much time and energy convincing his peers (Réaumur, 1741; Trembley, 1744; Ratcliff, 2004, 2012). Later on it became clear that together with jellyfish, sea anemones

Abbreviations used in this paper: ECM, extra-cellular matrix; HA, head activation; HI, head inhibition; i-cells, interstitial cells.

***Address correspondence to:** Brigitte Galliot. Department of Genetics and Evolution, University of Geneva, 4047 (Sciences III) 4, Bvd d'Yvoy, CH-1205 Geneva Switzerland. Fax: + 41 (0)22.379.33.40. e-mail: brigitte.galliot@unige.ch - web: http://genev.unige.ch/en/users/Brigitte-Galliot/unit

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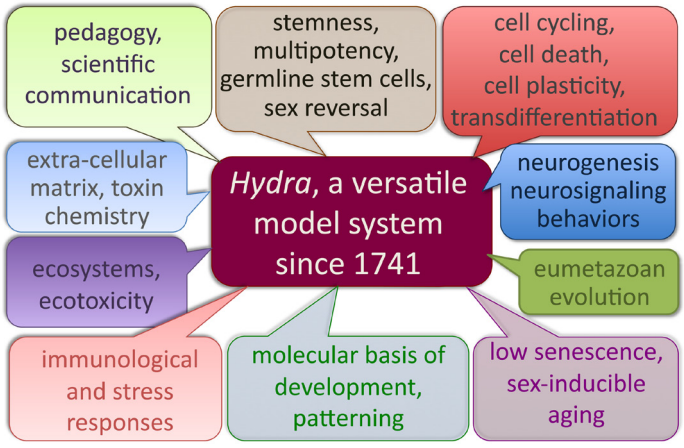

Fig. 1. The multiple aspects of biology that can be addressed thanks to the *Hydra* model system.

and corals, *Hydra* form a sister phylum to Bilateria named Cnidaria (Haeckel, 1896; Hyman, 1940; Collins *et al.*, 2006). In this issue Marc Ratcliff highlights a novel and influential aspect of Abraham Trembley's contribution to the development of experimental sciences. Indeed, with the accurate analysis of *Hydra* behaviors and tissues, he set up the tools and the conditions to investigate the mysterious laws of aquatic life in his study and no longer in the field, promoting thus the development of marine biology as an experimental, rather than solely observational, science (Ratcliff, 2012). This article is illustrated with historical drawings archived at the Public Library of Geneva, selected in 2010 for an exhibition entitled "*Abraham Trembley and the birth of marine zoology*".

Hydra a a model system to understand how developmental programs remain active or activable in adult organisms

In the late 19th century *Hydra* together with other hydromedusae emerged as fruitful model systems for physiology (Greenwood, 1888), cell biology (Weismann, 1883; Hadzi, 1909) and developmental biology (Rand, 1899; Tannreuther, 1908; Browne, 1909; Hyman, 1928). Indeed, *Hydra* polyps provided an experimental framework to study the mechanisms that regulate the maintenance of homeostasis even in extreme conditions (starvation, overfeeding) as well as those driving the reactivation of developmental programs after bisection or during budding (Fig. 1, Fig. 3).

Transplantation strategies to measure organizing activities and regenerative potential

Transplantation experiments performed on *Hydra* polyps were quite common at the turn of the 20th century. Ethel Browne, inspired by the recent results of her colleagues, developed a novel strategy that allowed her to discover "induction" (Browne, 1909) an essential principle in embryology. Although she never used the word "induction" herself, she understood and demonstrated the complexity of this process. First she cut a "*tentacle with a small bit of peristome (head tissue) at its basis*" from the donor and inserted it in a slit made along the body column of the host. In the following days she observed the development of an ectopic "hydranth" on the body column of the host, in 10 grafts out of 13 demonstrating, thus the robustness of the process (see her drawings reproduced in Bossert and Galliot in this issue).

Second, she wanted to trace the origin of the cells forming this ectopic "hydranth", and for that purpose she got the idea to perform transplantation between pigmented and depigmented *Hydra* of the same species. Indeed, a recent report had just identified a simple way to get rid of the "green bodies" of *Hydra viridis* (Whitney, 1907). That way she nicely demonstrated that the grafted tissue actually recruits cells from the host to form the ectopically developing structure and she wrote: *"From these experiments the conclusion must be drawn that it is principally the material of the body wall of the stock and not the hydranth material of the graft that forms the new hydranth"* (see Fig. 2). She also showed that in addition to the apical region of the polyp, the regenerating head and the presumptive head region in the growing bud do exhibit organizer activity.

This important work was published in 1909, 15 years before the report of Spemann and Mangold on the organizer activity of the dorsal lip of the amphibian embryo (Spemann and Mangold, 1924) Unfortunately Spemann who was aware of Ethel Browne's work and might have been inspired by her experiments, never cited her work (Lenhoff, 1991). Despite this lack of recognition, her transplantation approach opened an avenue and largely contributed to promote *Hydra* as a powerful model in developmental biology (Fig. 3).

Parallel Head activation (HA) and Head inhibition (HI) gradients along the body column

Because of the multiple types of grafting she tested, Ethel Browne understood that the grafted tissue provides the stimulus to develop an ectopic axis, whereas the cells of the host predominantly contribute to the formation of the induced structure, two criteria that fulfills the definition of an organizer (Fig. 2). However she did not figure out that the host might be able to inhibit the activity of the grafted tissue. Twenty years later the concepts of Head Activation and Head Inhibition emerged, represented as one pair of parallel gradients (Mutz, 1930; Child, 1932; Yao, 1945). Later on Gerald Webster and Lewis Wolpert in London, Harry MacWilliams in Worcester, and Tsutomu Sugiyama in Mishima, quantified these graded activities along the body column, showing maximal HA and HI levels at the apex (Webster, 1966a, b; Sugiyama, 1982; MacWilliams, 1983a, b; Takano and Sugiyama, 1983).

In the 1970s, with the aim of identifying the genetic basis of developmental mechanisms in *Hydra,* Toshitaka Fujisawa and Tsutomu Sugiyama decided to perform a screen to isolate *Hydra* mutants (Sugiyama and Fugisawa, 1977a, b; Sugiyama and Fujisawa, 1978a, b). Hiroshi Shimizu in this issue recapitulates the results they obtained by measuring precisely thanks to lateral transplantation experiments the slopes of the HA and HI gradients along the body column of a collection of *Hydra* strains isolated from the field or produced through breeding (Shimizu, 2012). To identify the cell types regulating HA and HI, Fujisawa and Sugiyama then applied the reaggregation technique developed by Richard Campbell (Marcum and Campbell, 1978) to produce chimeric animals made up of cell lineages isolated from highly different strains in terms of HA and HI slopes. With this sophisticated strategy they could thus deduce the role of each cell lineage and reached the conclusion that HI is primarily under the control of the endodermal epithelial cells with a slight modulation by the interstitial cells, whereas the HA gradient is directed by the ectodermal epithelial cells (Takano and Sugiyama, 1984; Shimizu, 2012).

Apical organizing activity versus self-organizing activity of the body column

A body of corroborating data obtained from lateral transplantation of tissues from the upper body column stresses that head formation, in this context, does not rely on organizer activity but rather on the self-organizing property of the grafted tissue (Yao, 1945; MacWilliams, 1983b; Broun and Bode, 2002). The main difference is the recruitment of host cells by the grafted tissue, and indeed the analysis of the tissues of the ectopic axis shows that upon grafting of the upper body column, the ectopic *Hydra* contains predominantly cells from the donor and not from the host, proving the low inductive activity of the graft. This is in sharp contrast with the results obtained when tissues from the hypostome, the head-regenerating tip or the growing bud are grafted (see above). The transient contact between a hypostome and the body column of the host actually suffices to induce an ectopic axis, suggesting that signaling molecules (morphogens) are released during the period of contact (Broun and Bode, 2002). Wnt3, the ligand of the canonical Wnt pathway appears as the signal that sets up the organizing activity in the head (Broun *et al.*, 2005; Gee *et al.*, 2010). In this issue, Hans Bode details the criteria that define the head organizer and discusses the molecular mechanisms that might distinguish induction from self-organization (Bode, 2012).

Theoretical modeling of the principles of developmental biology in Hydra

The tentacles immutably organized as a ring in *Hydra* likely inspired Alan Turing as he took this example of patterning, among others, to develop his model of reaction-diffusion and propose it as a model for the chemical basis of morphogenesis (Turing, 1952). This model of reaction-diffusion was itself used to develop several models describing positional information in *Hydra,* taking into accounts the two pairs of activation and inhibition gradients that had been characterized experimentally (Wolpert, 1969; Gierer and Meinhardt, 1972). With the idea of identifying general principles for development, i.e. to translate the genetic information into patterns, Lewis Wolpert considered hydroid regeneration, sea urchin gastrulation, epidermis patterning in insects and chick limb development to propose the unifying concept of positional information, whereby each cell receives an address corresponding to its position in a developing tissue/organ, i.e. in a co-ordinate system defined by reference points and boundaries (Wolpert, 1969). If *Hydra* is considered as a unipolar system, then the reference point for any cell along the body column would be the hypostome and a graded distribution of substances from the hypostome to any cell along the axis would provide this positional information. In fact there are two poles in *Hydra* and this positional information is supposed to be regulated by two pairs of linear parallel gradients, running opposite of each other, one for head activation / head inhibition and the second for foot activation / foot inhibition.

Few years later Alfred Gierer and Hans Meinhardt reinterpreted the *Hydra* transplantation data produced in the laboratory of Lewis Wolpert and deduced a molecular theory of biological pattern formation based on autocatalysis and lateral inhibition that integrated the concepts developed by Turing first and later by Wolpert (Gierer and Meinhardt, 1972). In short they proposed a non-linear activation / inhibition model where the cross-talk between two types of substances would drive morphogenesis, activators that act locally and self-enhance their activity (auto-catalytic), inhibitors that repress activators (cross-catalytic) over long-range distance. If one assumes that these two types of molecules are distributed in the tissue with different concentrations and different source densities, then this activation-inhibition model, which is based on non-linear interactions, would support the two phases of morphogenesis, a

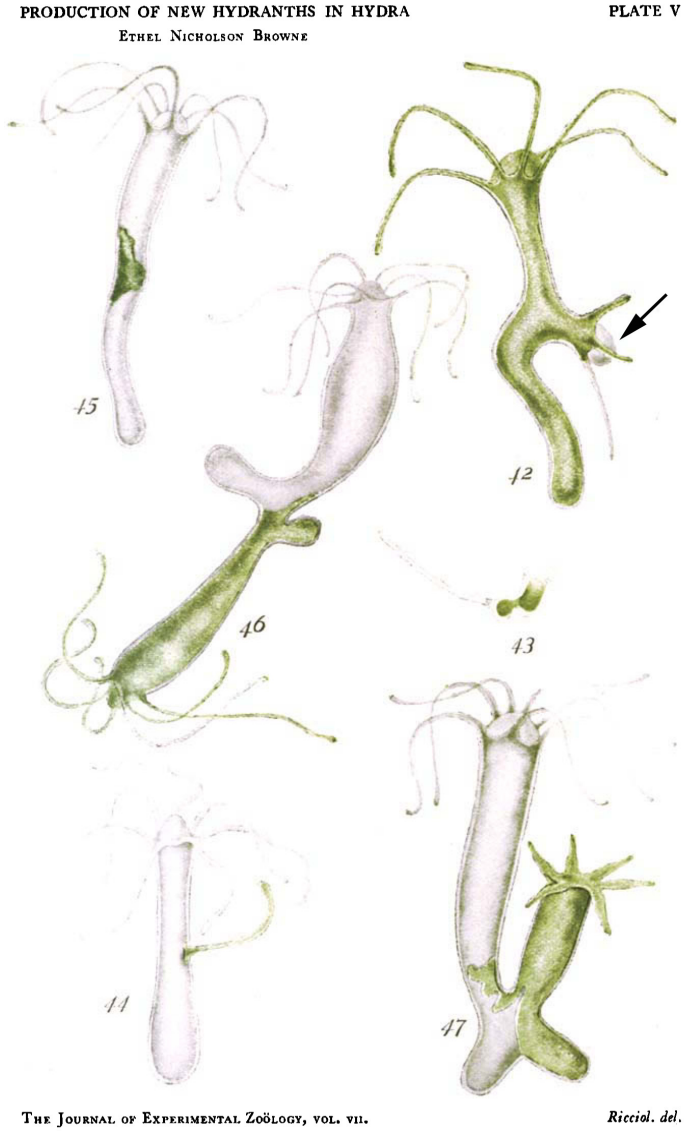

Fig. 2. Representative transplantation experiments performed by Ethel Browne between pigmented and depigmented *Hydra* polyps. *To depigment* Hydra viridis *that contain symbiotic algae (green), the polyps are treated with 0.5% glycerine for three weeks to become "artificial white hydra". All grafts were performed laterally at mid-body level, except when specified.* **(Exp 42)** *White tentacle with base (arrow) grafted on a green hydra showing the formation of an ectopic hydra with an important contribution of the host cells.* **(Exp 43)** *White tentacle with base grafted in the foot of a green hydra resulting in the formation of a minute hydranth.* **(Exp 44)** *Green tentacle without base grafted into a white hydra showing the lack of inducing activity and the absorbtion of the grafted tissue.* **(Exp 45)** *Graft of a ring of green tissue (body column) that remains as a patch, with no inducing activity.* **(Exp 46)** *Graft of a complete green hydra on a white host showing that the green hydra keeps its individuality and gets displaced towards the basal pole.* **(Exp 47)** *Basal half of a green hydra grafted by its apical side at mid-body level on a white hydra; after 6 days this basal half has regenerated a head and is close to detach from the host.*

fast one to establish a primary pattern (for example the organizing activity developed in few hours in the head-regenerating tip) and a slower one to differentiate the definitive structure. In case of *Hydra*, this model explains how de-novo patterns can arise, how any part of the body column can regenerate a complete animal.

In this issue, Alfred Gierer tells us how he moved from physics to microbiology, then to developmental biology, selecting the *Hydra* model system to develop concepts unifying the principles of development that can be applied to organisms more complex than *Hydra*, and finally to neurobiology (Gierer, 2012). He also discusses the role of mathematics to understand how cells generate real shapes, like stable cell sheets or evaginated structures. In the following review, Hans Meinhardt provides more experimental data that support the activation-inhibition model (Meinhardt, 2012), as the work of Ueli Technau *et al.,* who showed on regenerating aggregates that activation concerns about 10 to 15 cells, i.e. a 100 μm large region, whereas inhibitory activity extends over a 800-900 μm distance (Technau *et al*., 2000). At the molecular level, Wnt signals (specially Wnt3) fulfill the requirements of an activator, however inhibitor molecules that would fit the model remain to be identified in *Hydra*. In developing vertebrates, some activator/inhibitor couples such as Nodal/Lefty, involved in mesoderm formation and left/right patterning, fit well with the activation-inhibition model, indicating that indeed this model support developmental processes in eumetazoans.

Hydra - *a model system for aging studies*

An initial study on aging in *Hydra* was performed by Paul Brien in Paris who wanted to investigate the crosstalk between the asexual and sexual modes of reproduction (Brien, 1953). In two species, *Hydra vulgaris* and *Hydra viridis*, Brien noted that budding persists even in animals that undergo sexual differentiation; he also recorded that these animals did not seem to loose their fitness when surveyed over several years. In case of *Hydra oligactis* the result was different, animals exposed to cold induce their sexual differentiation, then rapidly stopped budding and after having laid eggs, become "exhausted" after three months and die. Although Brien did not use the words senescence or aging, two main conclusions could be deduced from his observations: 1) *Hydra* polyps maintained at room temperature exhibit no or very limited senescence, 2) *Hydra oligactis* that undergo sexual differentiation upon cold induction, rapidly die, providing thus a system where aging is inducible. These two aspects were indeed confirmed by independent studies: first the lack of senescence was tested in North America on cohorts of asexual *Hydra vulgaris* by Martinez (1998), second the inducibility of aging in *Hydra oligactis* was reproduced in Japan by Yoshida *et al*. (2006). In this issue Daniel Martinez and Diane Bridge discuss the role of proteins involved in the cellular stress response that are required to maintain homeostasis over a long term (Martinez and Bridge, 2012).

Hydra - a model system for stem cell biology

The biology of stem cells in* Hydra: *multipotency, sex determination and stemness

Hydra provides unique experimental conditions to investigate the biology of stem cells. Three distinct populations of continuously proliferating stem cells were characterized, epithelial endodermal and epithelial ectodermal stem cells that are each unipotent, i.e. providing epithelial cells that acquire specific features in the apical and basal regions. These epithelial stem cells that cannot replace each other, form two epidermal / gastrodermal sheets linked together by the extra-cellular matrix (named mesoglea) (Wood, 1961; Lentz, 1966; Sarras, 2012). The third stem cell population named interstitial stem cells, already identified as progenitors of the nematocyte lineage by Brauer (Brauer, 1891), actually provide progenitors for all cells of the nervous system, including the mechanosensory cells (named nematocytes or cnidocytes), but also for the gland cells of the digestive tract and for the germ cells when the animals follow the sexual cycle (Tardent, 1954; Brien and Reniers-Decoen, 1955; Burnett and Diehl, 1964; Diehl and Burnett, 1964; David and Murphy, 1977; Sugiyama and Fujisawa, 1978a; Bode *et al*., 1987; Bosch and Davis, 1987; Bode, 1996). This means that the interstitial stem cells provide both somatic cell lineages and germ cells all along the life of the animal. Their self-renewal property was proven in cloning experiments performed on aggregates made of cells treated with nitrogen mustard that are no longer able to proliferate, and in transplantation experiments performed on "epithelial" *Hydra* that no longer contain interstitial cells. In this issue three articles review our current knowledge about stem cells in *Hydra*, the first one by Charles David on the multipotency of the interstitial cells (David, 2012), the second by Chiemi Nishimiya-Fujisawa on the germline stem cells and the sex determination in *Hydra* (Nishimiya-Fujisawa and Kobayashi, 2012) and the third one by Bert Hobmayer and colleagues who discuss the stemness-related genes that might help distinguish between these three stem cell populations (Hobmayer *et al*., 2012).

The* Hydractinia *model system, a close cousin but marine and colonial

Sexual development is not easily amenable to experimentation in *Hydra* and marine cnidarian organisms that provide an inducible system for sexual development always offered valuable experimental alternatives (Frank *et al*., 2001; Galliot and Schmid, 2002; Houliston *et al*., 2010). One of the best examples is the marine colonial hydrozoan *Hydractinia* used by August Weizmann to identify the germ plasm that he distinguished from the somatic stem cells. Weizmann thus proposed the germ plasm theory whereby only germ cells and not somatic cells contribute to the transmission of characters (Weismann, 1883). Today *Hydractinia* is a fruitful model system for stem cell biology as seen by the recent elegant work done by Millane *et al.,* who ectopically expressed an Oct-4 related protein named Polynem in the epithelial cells and thus triggered the formation of neoplasm by reprogramming these epithelial cells to pluripotent interstitial cells (Millane *et al*., 2011). In this issue, Günter Plickert, Uri Frank and Werner Müller discuss the strength of the *Hydractinia* model system, not only for deciphering the mechanisms establishing stemness and cell reprogramming, but also, for addressing the role of Wnt signaling in developmental processes as well as the evolution of histo-compatibility (Plickert *et al*., 2012).

Cell to tissue signaling and *vice-versa*

Cell culture has never been successfully established from any cnidarian organism thus far. However, *Hydra* provides an experimental model system where cell behavior can be monitored in the context of an intact adult tissue. Indeed, *Hydra* is an organism that behaves as a whole, that can reactivate developmental programs

in a variety of contexts and that can now be manipulated so that as any cell lineage can be labeled thanks to the transgenic procedures recently established in the lab of Thomas Bosch (Wittlieb *et al.*, 2006; Khalturin *et al.*, 2007; Siebert *et al.*, 2008). All these properties would be lost in cell culture, which thus no longer appears essential compared to the advantages provided by a 4D physiological and developmental system, and the possibility anyhow to test in heterologous systems the molecular properties of the *Hydra* genes.

***Setting up the boundaries in adult and developing* Hydra**

In this issue Angelika Boettger and Monika Hassel provide arguments to uncover in *Hydra* some robust eumetazoan innovations that allow to set up boundaries between different morphogenetic fields (Bottger and Hassel, 2012). Firstly, and despite its apparent

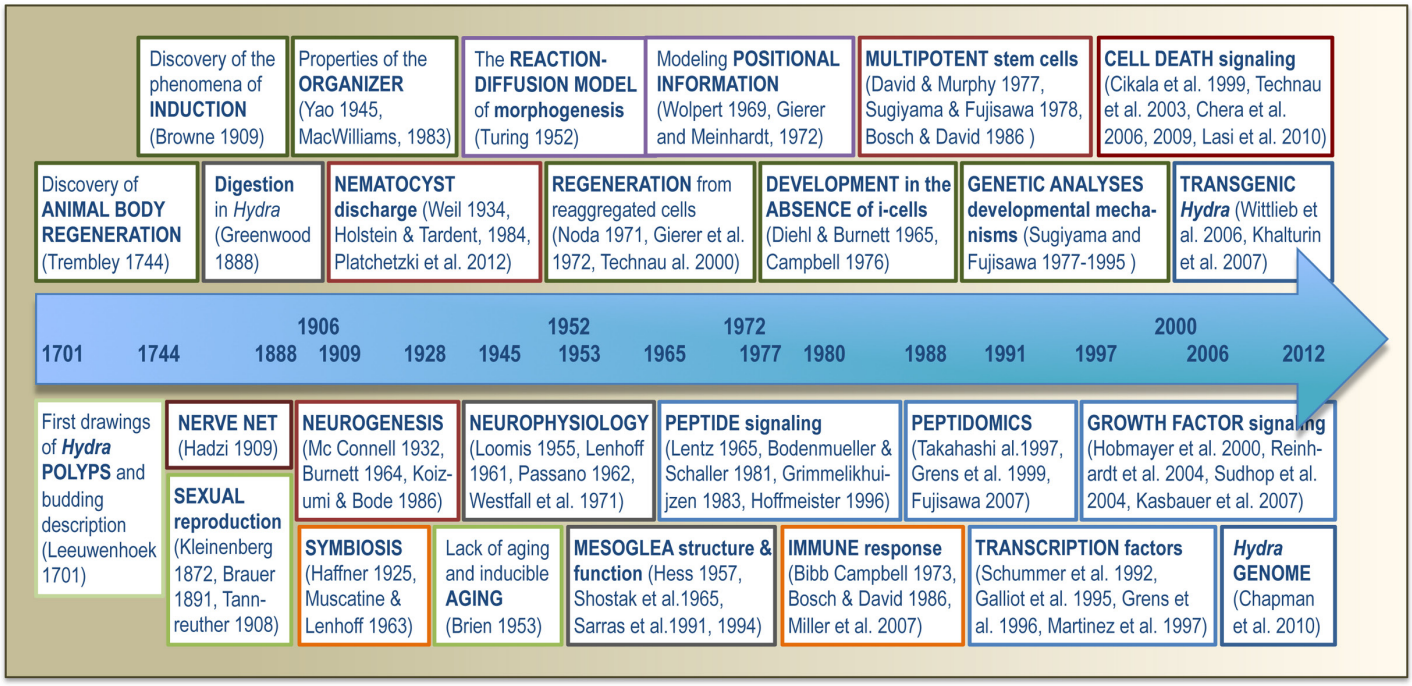

Fig. 3. Time line overview of the 300 first years of *Hydra* research. *This timeline lists key findings in* Hydra *research and the author apologizes for not referring to more findings, due to space constraints. See the indicated authors for a more complete reviewing of the following topics:* ***the first microscopic view of* Hydra *and budding*** *(Palm, 1996; Tannreuther, 1908; Hyman, 1928; Otto and Campbell, 1977a; Bottger and Hassel, 2012);* ***the discovery of* Hydra *regeneration and its scientific impact*** *(Trembley, 1744; Lenhoff and Lenhoff, 1986; Gierer, 2012; Ratcliff, 2012);* ***digestion*** *(Greenwood, 1888; Chera* et al., *2006; Sher* et al., *2008; Rachamim and Sher, 2012);* ***sex determination, sex reversal and embryogenesis*** *(Kleinenberg, 1872; Brauer, 1891; Hertwig, 1906; Tannreuther, 1908; Goetsch, 1922; Hyman, 1928; Loomis, 1954; Zihler, 1972; Sugiyama and Fugisawa, 1977a; Martin* et al., *1997; Nishimiya-Fujisawa and Kobayashi, 2012);* ***tissue induction and organizing activity*** *(Browne, 1909; Yao, 1945; MacWilliams, 1983a,b; Lenhoff, 1991; Broun and Bode, 2002; Bode, 2012);* **regeneration from reaggregated cells** *(Child, 1928; Noda, 1971; Gierer* et al., *1972; Murate* et al., *1997; Technau* et al., *2000);* ***the paradigmatic value of i-cell free (i.e. epithelial)* Hydra** *(Diehl and Burnett, 1964; Campbell, 1976; Sugiyama and Fujisawa, 1978a);* ***genetic analyses of developmental mechanisms*** *(Sugiyama and Fujisawa, 1977a, b; Marcum and Campbell, 1978; Sugiyama and Fujisawa, 1978a, b; Shimizu, 2012);* ***modeling of patterning*** *(Turing, 1952; Wolpert, 1969; Gierer and Meinhardt, 1972; Wolpert* et al., *1972; Gierer, 2012; Meinhardt, 2012);* ***stem cells and stemness*** *(David and Murphy, 1977; David and Plotnick, 1980; Bosch and David, 1987; Bode, 1996; Millane* et al., *2011; David, 2012; Hemmrich* et al., *in press; Hobmayer* et al., *2012; Plickert* et al., *2012);* ***nematocyte differentiation*** *(David and Gierer, 1974; Campbell and Marcum, 1980; Fujisawa* et al., *1986; Grens* et al., *1995; Lindgens* et al., *2004; Miljkovic-Licina* et al., *2004; Hwang* et al., *2007; Galliot* et al., *2009);* ***nematocyst structure, content and discharge*** *(Chapman and Tilney, 1959; Holstein and Tardent, 1984; Tardent, 1995; Beckmann and Ozbek, 2012; Plachetzki* et al., *2012; Rachamim and Sher, 2012);* ***anatomy and differentiation of the nervous system*** *(Schneider, 1890; Hadzi, 1909; McConnell, 1932; Burnett and Diehl, 1964; David and Gierer, 1974; Berking, 1979; Koizumi and Bode, 1986; Koizumi* et al., *1992; Gauchat* et al., *1998; Lindgens* et al., *2004; Guder* et al., *2006; Koizumi, 2007; Miljkovic-Licina* et al., *2007; Galliot and Quiquand, 2011);* ***neurophysiology*** *(Loomis, 1955; Lenhoff and Bovaird, 1961; Passano and McCullough, 1962, 1963; Westfall* et al., *1971; Kass-Simon and Pierobon, 2007; Pierobon, 2012);* ***aging and stress response*** *(Brien, 1953; Martinez, 1998; Yoshida* et al., *2006; Martinez and Bridge, 2012; Quinn, 2012);* ***mesoglea structure and functions*** *(Hess, 1957; Shostak* et al., *1965; Sarras* et al., *1991, 1994; Sarras and Deutzmann, 2001; Shimizu* et al., *2002; Sarras, 2012);* ***symbiosis*** *(Haffner, 1925; Muscatine and Lenhoff, 1963; Bossert and Dunn, 1986; Kovacevic, 2012);* ***innate immune system*** *(Bibb and Campbell, 1973; Bosch, 1986; Miller* et al., *2007; Altincicek and Vilcinskas, 2008; Bosch* et al., *2009; Augustin and Bosch, 2011),* ***peptide signaling and peptidomics*** *(Lentz, 1965; Bodenmuller and Schaller, 1981; Grimmelikhuijzen, 1983; Schaller* et al., *1989; Leitz* et al., *1994; Hoffmeister, 1996; Takahashi* et al., *1997; Grens* et al., *1999; Fujisawa, 2008; Fujisawa and Hayakawa, 2012);* ***transcription factors*** *(Schummer* et al., *1992; Galliot* et al., *1995; Grens* et al., *1996; Martinez* et al., *1997; Gauchat* et al., *1998; Broun* et al., *1999; Smith* et al., *1999; Technau and Bode, 1999; Gauchat* et al., *2000; Gauchat* et al., *2004; Lindgens* et al., *2004; Miljkovic-Licina* et al., *2007; Bridge* et al., *2010; Nakamura* et al., *2011; Ambrosone* et al., *2012; Klingel* et al., *2012);* ***cell death signaling*** *(Cikala* et al., *1999; Technau* et al., *2003; Chera* et al., *2006; Chera* et al., *2009; Lasi* et al., *2010a, b; Chera* et al., *2011; Reiter* et al., *2012);* ***growth factor signaling*** *(Bosch* et al., *1995; Hobmayer* et al., *2000; Kaloulis* et al., *2004; Philipp* et al., *2005; Arvizu* et al., *2006; Bosch, 2007; Bottger and Hassel, 2012; Galliot, 2012; Plickert* et al., *2012);* ***transgenesis*** *(Wittlieb* et al., *2006; Khalturin* et al., *2007; Dana* et al., *2012) and finally,* ***genomic, transcriptomic and proteomic studies*** *(Hwang* et al., *2007; Chapman* et al., *2010; Hemmrich* et al., *in press; Balasubramaniam* et al., *2012; Steele, 2012).*

simple and continuous shape, molecular markers as a series of gene expression patterns help visualize boundaries all along the body column of the adult *Hydra* polyp (Galliot, 2000; Hobmayer *et al*., 2000; Steele, 2002; Bottger and Hassel, 2012). Secondly, they argue that the formation of these boundaries can easily be monitored during budding, the asexual mode of reproduction preferentially used by *Hydra* when maintained in favorable conditions, i.e. at room temperature with regular feeding (Hyman, 1928; Brien, 1953; Otto and Campbell, 1977b, a). The budding process consists in the elongation of a new axis perpendicular to the parental one, it takes place in the lower part of the body column and produces a fully developed new *Hydra* ready to detach from the parent in few days. So far 17 molecular markers of the budding process have been identified and the analysis of their temporal and spatial regulation demonstrates the progressive regionalization of the growing bud, first to define the circular zone on the parent where the bud will form, then to define the bud spot from which the bud will emerge, and progressively the different domains in the growing bud that will get refined during bud maturation. Interestingly these markers point to highly conserved signaling pathways, Wnt, BMP, FGF and Notch. Pharmacological approaches have confirmed the importance of the FGFR/Notch signaling to definitely establish the parent/but boundary as when the FGFR or Notch patways are inhibited, buds develop well but never detach from the parent. Thus *Hydra* offers here a powerful and easily amenable experimental framework to investigate the intimate mechanisms of boundary formation.

Evolutionary studies in the genomic era

In 2010 the genome of *Hydra magnipapillata* was made available (Chapman *et al*., 2010) and in this issue Rob Steele asks three questions to clarify the content of these genomic sequences: What genes are present? What genes are absent? What genes are novel? (Steele, 2012). Basically all signaling pathways active in developing bilaterians can be found in the *Hydra* genome as anticipated from previous studies. In some cases the gene families even show an astonishing diversification. For example with respect to the Wnt signaling molecules, *Hydra* expresses 9 Wnt orthologs out of the 13 gene families found in bilaterians, when humans express 12 of them, *Anopheles* 6, *Drosophila* 5 and *C. elegans* a single one (Lengfeld *et al*., 2009). This analysis (and others) prove that ecdysozoans have lost a significant number of gene families that are conserved from cnidarians to vertebrates. Rob Steele also tells us what is missing in *Hydra;* transcription factors as Emx or Evx although present in other hydrozoan species, pluripotency regulators as Nanog and Klf4 that appear to be vertebrate innovations, and more surprisingly peptides previously characterized in *Hydra* either biochemically as Head Activator (Schaller & Bodenmueller, 1981) or molecularly as Heady (Lohmann and Bosch, 2000), both involved in apical patterning and bud formation. The lack of genes coding for short peptides might reflect the incomplete assembly of the genome or alternative biosynthetic pathways. Finally, this review proposes to visit some interesting perspectives that genomics and transcriptomics, combined with transgenic strategies make possible.

Peptide signaling in Hydra

First evidences for a putative signaling function of peptides in *Hydra* biology came in the early 1960s when efforts were made to extract from *Hydra* tissues substances that show a graded distribution along the body axis and would be able to affect head patterning. In addition, one expected such substances to be produced by nerve cells, as Lentz had shown that neurosecretory granules can induce the formation of multiple heads (Lentz, 1965). Protease-sensitive substances, whose activity was quantified by the number of tentacles regenerated per head, were indeed isolated and shown to localize in nerve membranes (Lesh and Burnett, 1964, 1966; Schaller and Gierer, 1973; Schaller, 1973). This Head Activator factor was shown to be active at very low concentration, thus ruling out possible unspecific contaminants as toxins from nematocysts (Muller and Spindler, 1971). Once purified in parallel from *Hydra*, sea anemone, human hypothalamus, rat hypothalamus, bovine intestine this 11mer peptide surprisingly showed an identical sequence highlighting for the first time the conservation of signaling molecules form cnidarians to mammals (Bodenmuller and Schaller, 1981; Schaller and Bodenmuller, 1981). Head Activator was then used in a number of studies by different groups and it turned out that, depending on its concentration, it can promote cell proliferation and neuronal differentiation, enhancing thus head regeneration and budding (Schaller *et al*., 1989; Schaller *et al*., 1996). As discussed by Rob Steele the corresponding gene could not be identified in the genome sequences (Steele, 2012). In parallel efforts, peptides promoting foot differentiation were purified by Sabine Hoffmeister, confirmed this time by gene cloning (Hoffmeister, 1996; Grens *et al*., 1999; Hoffmeister-Ullerich, 2007).

But peptides are not only active as morphogens as several classes of peptides actually perform neurophysiological tasks, and for a while neurotransmission was even assumed to be predominantly peptidergic in cnidarians (Grimmelikhuijzen *et al*., 2002; Pierobon, 2012). In the 1990s an ambitious screen for *Hydra* peptides was launched in Japan, identifying both neuropeptides and epitheliopeptides (Takahashi *et al*., 1997). In this issue, Toshitaka Fujisawa and Eisuke Hayakawa report about the signaling pathways activated by these peptides, most frequently through G-protein coupled receptors. They also discuss the highly variable evolutionary constraints applied on these peptide gene families, either conserved across eumetazoan phyla, or taxon-restricted, performing phylum- or even species-specific functions (Fujisawa and Hayakawa, 2012). Thus peptide analysis provides a tool to understand the evolution of *Hydra*-specific traits.

Hydra *- a model system for neurophysiology*

Hydra provides a model system for neurophysiological studies since the first description by Abraham Trembley of their active feeding behavior, immobilizing their food with their tentacles and ingesting it in a co-ordinated fashion in their mouth. 150 years later Jovan Hadzi described for the first time their neuroanatomy, sensory nerve cells connected to a loose neuronal network – nerve net- (Hadzi, 1909) and some years later Carl McConnell observed the development of the ectodermal nerve net when the animals differentiate their head (McConnell, 1932). Subsequently the feeding response was the topic of much attention and the fact that animals can feed on live animals but not on dead ones suggested that they received from their preys some signals necessary to initiate the feeding response (Beutler, 1924). Helen Park observed that reduced glutathione (GSH) induces a prolonged mouth opening and William Loomis and Howard Lenhoff proposed that GSH be the signal regulating the feeding behavior (Loomis, 1955; Lenhoff and Bovaird, 1961). However GSH might not be the only active substance (Forrest, 1962). Nevertheless GSH was subsequently

used as an exogenous stimulant to investigate the mechanisms of the feeding response independently of the nematocyst discharge. In parallel neurophysiologists identified the pacemakers that control behaviors in *Hydra* and characterized polarized synapses (Passano and McCullough, 1963; Westfall *et al*., 1971).

More recent pharmacological and molecular studies have demonstrated the co-existence in cnidarian nervous systems of fast (acetylcholine, glutamate, GABA, glycine) and slow (catecholamines, serotonin) neurotransmitters besides neuropeptides (Kass-Simon and Pierobon, 2007). In this issue, Paola Pierobon proposes a scenario whereby the multipolar GABAergic neurons in the apical region provide an integrative response that regulates the initiation and the termination of the feeding response (Pierobon, 2012).

The nematocyst, a sophisticated organelle with outstanding biomechanical properties

The nematocysts (or cnidocysts) are highly specialized venom-containing organelles, basically thick wall capsules equipped with a tubule, which differentiate in the nematocytes (or cnidocytes) and play a role in predation, in defence and in locomotion. Indeed, their discharge is responsible for the immobilisation of the preys, but also for the attachment of the tentacles to the substrate during walking (Ewer, 1947; Tardent, 1995). Since their initial description (Weill, 1934a, b), the study of their biogenesis and their physiology led to surprising discoveries as for example the unique speed of their discharge (Holstein and Tardent, 1984) or the negative regulation of their discharge by opsins (Plachetzki *et al*., 2012). In this issue Anna Beckmann and Suat Ozbek recapitulate what is currently known about their structural composition to provide these outstanding biomechanical properties. Interestingly they show that numerous constituants are actually found in the extra-cellular matrix (ECM) as collagens, lectins, glysominoglycans, as confirmed by the proteome analysis (Balasubramanian *et al*., 2012). They propose that to store safely soluble toxins, cells chose to import intracellularly ECM components (Beckmann and Ozbek, 2012).

The importance of the extra-cellular matrix (ECM) in developmental processes

The ECM in *Hydra* is a porous collagenous layer that maintains together the two epithelial cell layers, providing thus the shape but also the resistance and the flexibility of the animal (Sarras and Deutzmann, 2001; Shimizu *et al*., 2008). The questions raised by Michael Sarras in this issue are centered on two main issues: what are the biochemical properties that provide this unusual flexible and elastic structure, and what function(s) is playing the ECM on the amazing developmental potential of *Hydra* (Sarras, 2012). For the first question, he shows that the structure of the ECM is both conserved, sharing common structural elements with vertebrates as the laminin – collagen type IV polymerized network to provide the basal plasma membrane border of each epithelial layer (possibly interacting with epithelial cells through integrins), but also derived as the *Hydra* collagen type IV proteins, that show a quite distinct homotrimeric organization, much more flexible than the vertebrate collagen type IV. Similarly the major triple helical domain of the fibrillar collagen 1 has the same length in *Hydra* and in vertebrates but again the supramolecular organization is quite different with the *Hydra* Hcol-1 forming fine fibrils and the vertebrate collagen 1 rather forming banded fibrils.

Concerning the functions of the ECM, it was tested with multiple approaches (pharmacological, blocking antibodies, gene silencing via antisense RNA or insertion of exogenous ECM) in a variety of contexts, especially during reaggregation from dissociated tissues when the two epithelial cell layers come into contact in the absence of any ECM (Kishimoto *et al*., 1996; Murate *et al*., 1997), but also during regeneration and budding (Aufschnaiter *et al*., 2011). It turned out that the ECM plays an essential role for all these processes that are blocked when the ECM cannot form properly (see in Sarras, 2012). At the cellular level the ECM is required for cell proliferation, cell migration, cell differentiation, cell transdifferentiation. Future steps will then be to understand the bidirectional signaling that regulates the interactions between the epithelial cell layers and the ECM.

The homeostatic and developmental functions of cell death

Cell death occurs in multiple contexts in *Hydra*, first recognized in animals exposed to colchicine (Campbell, 1976), then in animals of the thermo-sensitive strain sf-1 submitted to heat-shock (Marcum *et al*., 1980), also in animals submitted to starvation (Bosch and David, 1984), to wounding (Fujisawa and David, 1984), to hetero-grafting (Bosch, 1986), or undergoing oogenesis (Honegger *et al*., 1989), spermatogenesis (Kuznetsov *et al*., 2001), massive autophagy (Chera *et al.,* 2006) and finally in head-regenerating tips after mid-gastric bisection (Chera *et al*., 2009). In the meanwhile, parallel studies showed that the molecular cell death machinery is highly conserved from *Hydra* to bilaterians (Cikala *et al*., 1999; Lasi *et al*., 2010a, b). In addition, a number of cellular and biochemical tools are now available to characterize and quantify cell death in *Hydra* (Lasi *et al*., 2010b; Reiter *et al*., 2012). As discussed by Reiter *et al.* in this issue, *Hydra* offers a unique model system to dissect the multiple regulation and functions of cell death in an adult organism; that is, to maintain homeostasis in the absence of nutrients, to react to stress and injury, to insure stem cell renewal and germ cell production, to participate in immune responses (Reiter *et al*., 2012).

Ecotoxicology and Environment

Hydra*, a model system for aquatic ecotoxicological studies*

Hydra is not only a model system for cell and developmental biology, but thanks to multiple endpoints that can be monitored (i.e. the morphology, the attachment, the feeding response, the growth rate, the regenerative response), *Hydra* is also a highly suitable model system for aquatic ecotoxicological studies. *Hydra* are highly sensitive to heavy metals (cadmium, zinc, copper, uranium, magnesium), possibly because they lack the metal-binding protein metallothionein. However Brian Quinn, François Gagné and Christian Blaise tell us that very little is known about the mechanisms that underlie this toxicity (Quinn *et al*., 2012). One possibility they discuss is to perform comparative analyses between *Hydra* species that show highly different levels of thermo tolerance as there is a cross protection between thermo tolerance and metal tolerance. *Hydra oligactis* that displays a reduced stability of hsp70 possibly explaining their lower thermo tolerance (Brennecke *et al*., 1998), would provide a well-suited framework to test tolerance to metals. But *Hydra* are also highly sensitive to endocrine disrupting compounds as bisphenol A, as evidenced by the rapid alterations of the tentacle morphology or the sexual reproduction. *Hydra* are good candidates to be tested to organophosphorus pesticides or

to drugs frequently present in municipal effluents as carbamazepine by looking at the cellular stress responses, at the oxidative stress. By contrast they exhibit a rather low sensitivity to organic toxicants (polychlorinated biphenyls, 4-chlorophenol, lindane, mirex,..). With the exception of organic toxicants, *Hydra* appears as one of the most sensitive species to toxicants when compared to other invertebrate test organisms, especially for sub-lethal effects. Moreover the use of symbiotic versus aposymbiotic animals can provide useful conditions for investigating the mechanisms of a detected toxicity.

Value of the* Hydra *model system for studying endosymbiosis

Unicellular dinoflagellate algae frequently inhabit aquatic invertebrates establishing with their host a mutualistic partnership named symbiosis. Among cnidarians the importance of endosymbiosis is well-established in reef-building corals (Weis and Allemand, 2009). However, endosymbiosis is not restricted to stony corals in cnidarians, it is also present in some sea anemones, jellyfish and *Hydra* species (see in Venn *et al*., 2008). Out of the four *Hydra* species (Kawaida *et al*., 2010; Martinez *et al*., 2010), only *Hydra viridissima* shows endosymbiosis with photosynthetic *Chlorella* algae living in the endodermal epithelial cells, and transmitted to the oocyte when the animals undergo sexual differentiation (Muscatine and Lenhoff, 1963; Rahat and Reich, 1985). More than 100 years ago Whitney found a way to eliminate these algae without compromising the survival of their host, which then become aposymbiotic (Whitney, 1907). As *Hydra* can be easily mass cultured in the laboratory, this procedure opened the way to study the complex interactions between the host and the symbionts and design strategies to identify the signals that allow the symbionts to invade the host and avoid its immunological response (Muscatine and Lenhoff, 1965). In this issue Goran Kovacevic recapitulates the current knowledge about the cellular and metabolic processes linked to endosymbiosis in *Hydra* and discusses the impact of gene flow between symbionts and *Hydra* (Kovacevic, 2012).

***The chemical arsenal and the chemical landscape of* Hydra**

In this issue Tamar Rachamim and Daniel Sher discuss the impact of *Hydra* on its environment, first considering the venom they produce in their nematocysts, but also taking into account all the bioactive compounds they release (Rachamim and Sher, 2012). The venom is supposed to be a complex mix up of toxic as well as non-toxic molecules that all together are responsible for the toxicity of a given venom. In case of *Hydra* the knowledge concerning the composition of the venom is currently quite vague and partial, although some components were characterized as some cytolytic actinoporins, some ShK domain proteins that block the K+ channels or the neurotoxic phospholipase A2 protein. All these toxic proteins are supposed to have diverged from non-toxic proteins, representing thus convergent evolutionary events between different venomous species. Moreover the composition of the venom is likely different between the different types of nematocysts, either involved in the feeding response (stenoteles, desmonemes), or in defence (holotrichous isorhizae) or in locomotion (atrichous isorhizae) (Ewer, 1947). Therefore further studies should be performed on purified homogenous populations of nematocysts.

As mentioned above, the chemical arsenal in *Hydra* extends outside nematocytes with bioactive compounds showing tissue or cell-type restricted expression, as the antimicrobial peptide *Periculin 1A* expressed exclusively in the female germline (Fraune *et al*., 2010), the paralytic and pore-forming *Hydralysin* genes expressed in the digestive cells (Sher *et al*., 2008), the *MAC-PF* genes encoding pore-forming domain proteins expressed in gland cells (Miller *et al*., 2007) or in the peduncle region (Amimoto *et al*., 2006). Tamar Rachamim and Daniel Sher proposes that together with the discharged venom, the overlap of these domains of expression create “chemical landscapes” along the *Hydra* body that can be sensed by the neighboring polyps when *Hydra* live in groups, or by the preys, or by any organism present in the surroundings. Hence *Hydra* might provide an experimental model to understand how bioactive compounds affect the aquatic biosystem.

Pedagogy

Hydra *- a model system for teaching science*

The last review of this issue focuses on the pedagogical value of the *Hydra* model system, initially demonstrated by Abraham Trembley who performed his experimental research with the two young children of Count Bentick. This review takes advantage of the unique expertise of Patricia Bossert in this matter, who, as a high school teacher and pedagogical consultant, continuously used the *Hydra* model system to teach biology in high school classes of New York State over the past 30 years (Bossert and Galliot, 2012). Together with dedicated students she developed a panel of tools and strategies to investigate a variety of biological questions that touch ecology, selection in evolution, photobiology, developmental biology and molecular biology. Each of the teaching units proposed in this article is designed to provide the basic protocols of precise experiments with expected results but also to challenge the students to go further and design their own experiments. Thus, we expect that thanks to the *Hydra* model system, teachers and young students will develop their curiosity, their creativity and their rationale thinking.

Perspectives

In the field of cell biology, some key questions remain unanswered. One of them is the degree of cell plasticity that can be observed in *Hydra*: these animals regenerate in the absence of cell proliferation or in the total absence of nervous system. Does it mean that cell proliferation and nerve cells are not used when the animals are fully equipped or does it instead mean that the animals reprogram when one of their attributes is missing? The second proposition would permit reconciliation of sets of data that at the moment appear contradictory, as for example the role of substances produced by the nerve cells that were shown to promote head formation and participate in morphogenetic processes. A better understanding of the limits and regulation of this plasticity is a major task for the coming years.

Similarly in the field of developmental biology regeneration is often considered as a simple reinterpretation or translation of the homeostatic parameters with no specific regenerative program. However, one can also consider that the reactivation step in the developmental program is in itself specific to regeneration and not simply derived from the homeostatic condition that precedes injury. A series of data actually suggest that this reactivation step is drastically constrained by the homeostatic context as evidenced by the studies that show significant differences between the early stages of head regeneration after decapitation (which is used as

the reference situation in numerous studies) or after mid-gastric bisection. Again *Hydra* provides a highly suited system to study the impact of the homeostatic context on the way to launch a regenerative program, a major question to solve before establishing regenerative strategies.

The aim of this issue was primarily to demonstrate to scientists and teachers at any stage of their career that the *Hydra* model system is fruitful, promising and potent. Potent because this model combines the simplicity of its anatomy to a sound basis of knowledge of the developmental and cellular processes; fruitful because the biological questions that can be addressed with the *Hydra* model system are multiple and become more and more diverse with time, and finally promising because this system now provides functional tools that can be associated to genomic, transcriptomic and proteomic approaches. Gene knockdown through RNA interference was first established by electroporation, a rather harmful procedure (Lohmann and Bosch, 2000). Later feeding animals with dsRNAs proved to be a relatively easy, incremental and highly efficient procedure when controled at the RNA and protein levels, producing a variety of well characterized phenotypes (Chera *et al*., 2006, Miljkovic-Licina *et al*., 2007, Chera *et al*., 2009; Chera *et al*., 2011). However the stability of its efficiency in the various *Hydra* strains/species seems to be affected by infectious agents that compete for the components of the RNAi machinery (BG, unpublished). Therefore additional efforts need to be done to characterize the agents interfering with the RNAi machinery in *Hydra*. To finish I would say that *Hydra* are simply esthetic and this is a great pleasure to see them everyday.

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