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Abstract

Over the past decades, genetic analyses performed in vertebrate and invertebrate organisms deciphered numerous cellular and molecular mechanisms deployed during sexual development and identified genetic circuits largely shared among bilaterians. By contrast the functional analysis of the mechanisms that support regenerative processes in species randomly scattered among the animal kingdom, were for long limited by the lack of genetic tools. Consequently unifying principles explaining how stress and injury can lead to the reactivation of a complete developmental program with restoration of original shape and function remained beyond reach of understanding. Recent data on cell plasticity suggest that beside the classical developmental approach, the analysis of homeostasis and asexual reproduction in adult organisms provides novel entry points to dissect the regenerative potential of a given species, a given organ or a given tissue. As a clue, both tissue homeostasis and regeneration dynamics rely on the availability of stem cells and/or on the plasticity of differentiated cells to replenish the missing structure. The freshwater Hydra polyp provides us with a unique model system to study the intricate relationships between the mechanisms that regulate the maintenance of homeostasis even in extreme conditions (starvation, overfeeding) and the reactivation of developmental programs after bisection or during budding. Interestingly head regeneration in Hydra can follow several routes according to the level of amputation, suggesting that indeed the homeostatic background dramatically influences the route taken to bridge injury to regeneration.

1. Introduction to adult developmental biology

A wide range of distinct biological processes contribute to the preservation of the anatomical form and functionality in adult animal organisms; these processes are acting at different levels, such as metabolism that affects the whole organism, cell turnover of organs and tissues, autophagy of specific cell types, DNA repair at the nuclear level (Rando 2006). As human beings we often consider that a high cell turnover is an obligatory rule to maintain the integrity of adult organisms. However, this is certainly not systematically observed across animal phyla as several species with short lifespan can be strictly post-mitotic after development, meaning that the differentiated cells can undergo cell growth but no proliferation during adulthood. The nematodes that keep their number of somatic cells constant in adulthood, provide the best example; similarly, in Drosophila all somatic adult tissues are post-mitotic except the gut. This drastic regulation of adult cell number generally impedes adult plasticity, which is required for homeostatic or regenerative mechanisms. However, in most metazoan species, the main way to protect adult organisms from physiological dysfunctions involves the removal and replacement of old or damaged differentiated cells. This ongoing physiological replacement process is named cell turnover. The adult stem cells (ASCs) play a key role in this turnover, although limited to the organ or the tissue where they reside (Wagers and Weissman 2004; Ohlstein and Spradling 2006; Blanpain et al. 2007). As a classical scenario, ASCs rather divide through asymmetric division, one of the daughter cell keeping the “stemness” status (self-renewal) whereas the second one, no longer a stem cell, undergoes a series of cell division, providing a transient amplifying stock that will subsequently commit to one or a series of differentiated fates (Nechiporuk et al. 2003). As a consequence three competitive processes regulate homeostasis: cell death, cell proliferation and cell differentiation. The study of their crosstalk in Drosophila imaginal discs showed how a coordinated cell-cell signaling tightly regulates this competition in a given tissue (Moreno and Basler 2004). In mammalian tissues, cell turnover occurs in epidermis, intestine, lung, blood, bone marrow, thymus, testis, uterus and mammary gland with large variations in the rate of cell turnover, from few days for the intestinal epithelium up to several months for the lung epithelium (Blanpain et al. 2007). In other organs (brain, heart, pancreas, kidney, cornea, …), the physiological cell turnover is likely limited and/or very slow, making difficult the in vivo monitoring of the respective behaviors of stem cells and dying cells.

Similarly to cell turnover, tissue repair also allows tissue replacement but requires the damage-induced activation of programs that monitor cell proliferation and cell differentiation. Finally, regeneration of anatomical structures like appendages, represent an
even more complex process with formation of a transient proliferative structure, the blastema, and activation of a developmental program that leads to restoration of original shape and function (Brookes and Kumar 2005). Both tissue repair and regeneration that affect different tissue types and require cell replacement on a large-scale, are triggered by nonspecific and usually exogenous damage, whereas cell turnover is a process that is endogenously initiated and restricted to a fraction of cells (Pellettieri and Sanchez Alvarado 2007).

Nevertheless one can intuitively perceive a progression from basic tissue self-renewal to tissue repair, reached by some but not all organs, to regeneration, accessed by a "happy few" elite of organs or structures. This view suggests a possible continuum between the processes that regulate each step, even though their complexity is supposed to gradually increase. To challenge the solidity of this view we propose here to review some results recently obtained in the paradigmatic Hydra model system. But before considering the different forms of plasticity deployed in Hydra, we will first discuss the origin and the current meaning of the concept of plasticity. Indeed this concept is widely used by biologists from different fields, but sometimes covering quite distinct meanings.

The ambiguities of the concept of "plasticity"

The word "plasticity" (from Latin plasticus or Greek plastikoς, ability to mold) refers to the "capacity of distortable bodies to change their shape under the action of an external force and to maintain the change after this force has ceased to act" (from Littre French dictionary, translated by (Will et al. 2008). At the first look, this definition apparently applies quite well to the regenerative process, however the usage of the word plasticity in biology is much broader, focusing on the ability of living organisms to adapt to constraints by changing their organization at a specific level, e.g. evolutionary, developmental, phenotypic, synaptic, cellular, molecular. As a consequence, the word "plasticity" should never be used alone but always be specified by the level where it applies (Pomerantz and Blau 2004). Some scientists even proposed to apply to the concept of plasticity in biological systems a more "engineer-oriented" usage, restricting it to the contexts where lasting structural reorganization, i.e. modifications of the material structure of the system (interface, connectivity network, constitutive elements), are indeed proven, leaving out of plasticity the effects of variability, flexibility, systematic variations and vicarious (substituted) processes as these effects rather result from "operational" than structural changes (Will et al. 2008). We selected here few examples to discuss this view, certainly more rigorous or at least less metaphoric (following the words of Will et al.) but as we will see difficult to apply in some contexts.

Evolutionary plasticity is certainly the best example of plasticity with structural changes leading to lasting changes. The combination of genomic, genetic and developmental approaches over the past 20 years have definitively proven that variations in the genomic organization of the Hox gene clusters obviously lead to genetic reprogramming during development and to species-specific modifications of the body plan (Duboule 2007). Developmental plasticity that was identified first in sea urchin embryos by Driesch in 1892 and later in vertebrate embryos, refers to the embryonic potential for regulation as the embryonic cells at early stages have the ability to change their fate to compensate for cell loss (Driesch 1900). This potential, which accounts for the occurrence of homozygous twins, is transient but can still be observed at later stages in more specialized tissues as limb buds (Summerbell 1981) or neural crest cells (Vaglia and Hall 1999). Developmental plasticity, more recently named transfating (Keleher and Stent 1990), requires the activation of the Gene Regulatory Network (GRN) that corresponds to the new cell fate. Interestingly in sea urchin embryo this activation apparently depends on inputs that are distinct during normal and regenerative developments (Ettensonho et al. 2007). If confirmed as a general rule, this would mean that context-specific signals sensed at the “interface” of the system induce long lasting structural reorganizations of the developing organism.

Phenotypic plasticity is “the property of a given genotype to produce different phenotypes in response to distinct environmental conditions” (Metcalfe 1906), with the first study of adaptive phenotypic plasticity described in the crustacean Daphnia. However the different phenotypes might reveal an intrinsic “repertoire of competences” that need no structural changes to be expressed (Will et al. 2008). In the same year, 1906, the term neuroplasticity was proposed by Ernesto Lugaro, a psychiatrist, who referred to the changes in neural activity during psychic maturation, learning processes or post-damage recovery (Berlucchi 2002). During the first half of the 1900s, the concept of brain plasticity was rejected by the scientific community, as it was unanimously accepted that the fully developed brain reached stability at adulthood, each region of the brain performing specific function(s) that could not be modified. In the 1960s, this view started to be challenged by experiments proving activity-dependent brain plasticity (Bennett et al. 1964; Bach-y-Rita et al. 1969). Synaptic plasticity, the capability for a neuron to modify on the long term its electrophysiological activity according to the stimuli it had received, was first studied in the mollusk Aplysia (Bruner and Tauc 1965; Kandel and Tauc 1965). The choice of this model system was instrumental to establish the importance of plasticity in the learning and memory processes as persistent modifications of the activity of the genetic circuitry are required to sustain changes in neurophysiological activity (Barco et al. 2006).

Cellular plasticity is directly related to the questions addressed in this review, i.e. what conditions of tissue homeostasis support a regenerative response. For this reason we will discuss here only the cellular plasticity of somatic cells (Figure 1). As a first but rather rare strategy differentiated cells can re-enter the cell cycle...
after injury, as exemplified by hepatocytes in mammals (Rabes et al. 1976). More frequently adult differentiated cells actually dedifferentiate upon injury before entering an active cycling phase to form a blastema (see below). But cells can also undergo metaplasia, i.e. phenotypically convert from one cell or tissue type into another, a process well known by pathologists, which actually covers a variety of processes.

Among those transdifferentiation is defined by the fact that stably differentiated cells irreversibly change their fate, i.e. reprogram by acquiring a novel differentiated status with a specific molecular signature (Okada 1991; Eguchi and Kodama 1993). During that process, the cells may or may not traverse the cell cycle. Similarly cell fusion that, as transdifferentiation is also increased upon injury, might lead to reprogramming when two distinct cell types fuse (Chiu and Blau 1984; Pomerantz and Blau 2004). Obtaining the experimental proofs of transdifferentiation is often difficult, but at least morphological and molecular criteria as well as cell lineage relationships should clearly characterize the two cell states before and after transdifferentiation (Slack 2007). In fact the most compelling evidence is provided by the transient co-expression of markers of the two differentiated cell states (Schmid and Alder 1984).

More recently it was possible to induce transdifferentiation by overexpressing one or several cell-specific transcription factors that suffice to convert one cell type to another (Slack 2007; Eberhard and Tosh 2008; Zhou et al. 2008). Indeed nuclear reprogramming plays an essential role in cellular plasticity and developmental biologists actually provided the first experimental evidences: they showed that nuclei isolated from mature somatic cells and transplanted into enucleated Xenopus oocytes, could reprogram and orchestrate the development of a frog (Gurdon et al. 1958). This surprising finding meant that nuclei of terminally differentiated cells can become totipotent. Forty years later the cloning of the sheep Dolly, also obtained by nuclear transfer from an adult somatic tissue, the mammary gland, confirmed this major finding in mammals (Wilmut et al. 1997). Actually even nuclei from post-mitotic neurons can be reprogrammed to drive the complete development of mice (Eggan et al. 2004).

Finally since 2006 reprogramming of mature somatic cells can be pushed to the point where adult differentiated cells directly reach an embryonic-like stemness thanks to the co-expression of defined transcription factors without using oocytes. Such cells, named induced pluripotent stem cells (iPSC), were obtained so far from fibroblasts (Takahashi and Yamanaka 2006; Takahashi et al. 2007), lymphocytes (Hanna et al. 2008), keratinocytes (Aasen et al. 2008), cord blood cells (Haase et al. 2009), smooth muscle cells (Lee et al. 2010). Whatever the procedure, reprogramming relies on epigenetic changes (Loh et al. 2008; Hochedlinger and Plath 2009), which certainly corresponds to “material” changes although not necessarily “structural” changes.

Two emerging model systems for investigating homeostasis and regeneration

Two historical invertebrate model systems, Hydra and planarians, were recognized since long to be suitable for investigating the mechanisms supporting tissue homeostasis, active maintenance of patterning in adulthood as well as complex cellular reorganization to regenerate after injury. The freshwater polyp Hydra that belongs to Cnidaria, a sister phylum to bilaterians, and the flatworm Planaria that belongs to Lophotrochozoa (see their respective phylogenetic positions in Figure 2) actually share five cellular and developmental features:

1) an intense and continuous tissue replacement in adulthood due to a stock of mitotically active stem cells, unique in case of planarians (the neoblasts), triple in case of Hydra (the ectodermal epithelial stem cells, the endodermal epithelial stem cells and the interstitial stem cells);
2) a stock of adult pluripotent stem cells that produce all along the life of the animals both germ cells and somatic cells (the interstitial stem cells in Hydra; the neoblasts in planarians), a situation quite unique among most animal phyla where germ cells usually segregate during early embryonic development;
3) an efficient asexual reproduction, through budding in Hydra and fission in Planaria;
4) the amazing property to regenerate almost any missing part of the body after injury;
5) an apparent lack of ageing, at least when the animals do not enter the sexual cycle (Martinez 1998; Yoshida et al. 2006; Pearson and Sanchez Alvarado 2008).

However Hydra and planarians are not genetically tractable. The recent development of genomic, molecular and cellular tools promoted their emergence as modern model systems where the mechanisms of homeostasis and regeneration can now be investigated thanks to RNA interference (RNAi) gene knocked down and transgenesis (Reddien and Sanchez Alvarado 2004; Galliot et al. 2006; Bosch 2007; Bottinger and Alexandria 2007; Salo et al. 2009). Homeostasis in planarians was recently reviewed in length (Pelletieri and Sanchez Alvarado 2007; Rossi et al. 2008; Handberg-Thorsager et al. 2008) and we will report here about the distinct forms of plasticity that take place in adult Hydra polyps, first
in response to variations in the feeding diet, and second after bisection, when the animal survives the amputation stress and regenerates the missing part. Given that most gene families that control cellular and developmental behaviors are present and highly conserved in cnidarians (Putnam et al. 2007; Chapman et al. 2010), these forms of plasticity are likely not exotic and we will discuss the correspondences between these changes and those observed in various bilaterian model systems.

2. Tissue plasticity in homeostasis

The Hydra body wall comprises two layers, ectodermal and endodermal, which all together contain about a dozen cell types. These cells derive from three distinct stem cell populations, ectodermal myoepithelial, endodermal myoepithelial, and interstitial cells (Dubel et al. 1987; Bode 1996; Steele 2002; Galliot et al. 2006). The spatial distribution of stem cells, progenitors and differentiated cells along the polyp occurs as a consequence of the continuous division of stem cells in the body column joined to the active migration or the passive displacement of the committed/precursor cells in either apical or basal directions (Figure 3A). According to the cell types, the cells terminally differentiate during their move or at their final position. Hence, the extremities, i.e. the tentacles, the hypostome (dome apex surrounding the mouth opening) and the basal disk, are made up of terminally differentiated cells that are continuously sloughed off. This permanent source of stem cells in the Hydra body column likely confers its unique cellular plasticity among multi-cellular adult organisms.

**Budding and autophagy, two ways to maintain fitness in Hydra**

In Hydra the homeostasis mechanisms tightly link cell renewal to an active maintenance of shape and fitness; this is best illustrated by the adaptation of Hydra to feeding conditions, when the animal regulates its steady state by growing and budding upon regular feeding. Upon starvation, the steady-state is no longer maintained and the animals degrow (Shostak 1974). Indeed in the laboratory where it is relatively easy to control the diet of a given population as Hydra are fed with freshly hatched Artemia nauplii, Hydra reduces its size but maintains its shape, fitness and regenerative potential over long periods of starvation (up to 4 weeks). Therefore numerous studies investigated the modulations of homeostasis in response to nutrient abundance, showing that the feeding diet dramatically influence the morphogenetic processes, namely the budding rate and the maintenance of patterning, as well as the cycling of the epithelial cells (Bode et al. 1977; Otto and Campbell 1977). Three distinct responses were characterized according to the level of feeding (Figure 3B, 2C). **In well-fed animals** (3 to 24 Artemia per day), the cell production exceeds tissue growth rate, and the cellular “surplus” is “eliminated” through asexual reproduction – a fast process, which causes the growth of a bud on the parental body, which itself does not grow. This budding process results in doubling the animal number each 2-4 days;
the distinct cell populations composing the tissue mass indeed increase but their relative proportions remain stable (David and Campbell 1972; Bode et al. 1973).

By contrast in over-fed animals (over 25 artemia per day), both cell proliferation and budding are increased (Otto and Campbell 1977; Bode et al. 1977) and the tissue mass exceeds the loss of tissue caused by budding. Considering that the tissue loss at the base and at the tentacles is comparatively low, the rate of budding predominantly regulates both tissue loss and the length of the parent’s body. Some reports actually suggest differences between the different Hydra species: the Japanese species H. magnipapillata elongates its body column while the head remains almost constant size (Kroiher 1999), whereas the European species H. vulgaris maintains its proportions (Müller 1995). However, the steady state is never reached in overfed animals, which will eventually

Figure 3. Cellular and morphological variations induced by the feeding diet in Hydra.
A) The homeostasis of the Hydra adult polyp relies on the dynamic equilibrium between cell gain and cell loss. Cell proliferation takes place in the body column whereas cells differentiate when they migrate or get displaced to the apical and basal poles, respectively the head region with tentacles and hypostome (dome surrounding the mouth opening) and the basal disc. Subsequently, these cells get sloughed off from the extremities and are replaced by the continuous influx of younger cells. B) The number of cells in a polyp is directly influenced by the feeding diet. Here the cell number by Hydra was plotted against the number of artemia given in the daily feeding (data taken from Bode et al., 1977). For each feeding period a two-degree polynomial function was calculated to show the tendency of cell number changes. C) Morphogenesis in Hydra is directly influenced by the feeding diet. Upon starvation the animals stop budding and rapidly activate autophagy to survive, reducing their size but keeping intact their morphology (red left panel). In steady-state condition, the animal size is stable and asexual reproduction, i.e. budding, takes place with new buds forming every 2 or 3 days (middle blue panel). In overfeeding conditions (green right panel), homeostasis is not maintained as heteromorphosis (bizarre morphological changes) precede the animal death (Otto and Campbell, 1977; Bode, 1977). Note that in all conditions bisection triggers regeneration.
undergo heteromorphosis (bizarre morphological changes) and die (Bode et al. 1977).

Finally, at low feeding level or under starvation conditions (0-1 artemia per day), Hydra polyps rapidly stop budding and progressively decrease their size to about half with no alteration of their body shape or their fitness (Figure 3C). Surprisingly, the relative sizes of the different cell populations, as well as the total number of cells per animal, remain almost constant (Bode et al. 1973). In fact, an imbalance between the decrease in polyp size and the cell cycle length was observed, as cell proliferation initially remains roughly constant, leading to the overproduction of 10% cells per day (Bosch and David 1984). This apparent contradiction was explained when Bosch and David found that apoptosis is actually rapidly induced upon starvation, in about 2 days (Figure 4). As a consequence the supernumerary cells produced by cell proliferation during starvation become apoptotic and are engulfed by the neighboring epithelial cells, providing a regulatory mechanism for keeping more or less stable the cell number (Bosch and David 1984; Pauly et al. 2007; Bottger and Alexandrova 2007).

However, the apoptotic process that affects less than 2% of the cells, remains stable over the starvation process (Bosch and David 1984; Chera et al. 2009a). Therefore, apoptosis is likely not sufficient for providing a long-standing energy support. More recently, autophagy was detected when feeding is stopped, first in the ectodermal, later on in the endodermal myoepithelial cells (Bugzarui et al. 2008; Chera et al. 2009a). But in contrast to apoptosis that remains constant during starvation, autophagy progressively affects all epithelial cells, providing hence a source of nutrients over long periods of starvation (Figure 4). Upon feeding resumption, the animals immediately stop autophagy, start to re-grow, and recover in several days their size and their ability to bud. Thus, the data currently available suggest that Hydra adapts to low feeding diet through two distinct cellular mechanisms, autophagy for energy support and apoptosis for cell number.

**Autophagy in Hydra leads to cell death when derepressed**

A second form of autophagy was actually discovered by pure serendipity when Kazal1, a Serine Protease INhibitor Kazal-type (SPINK) gene, was silenced by repeatedly feeding Hydra with dsRNAs. Progressively an excessive autophagy was observed in the endodermal cells of these intact Kazal1(RNAi) Hydra; large autophagosomes formed in the digestive cells, progressively fusing and leading to cell shrinkage and cell death in several days (Chera et al. 2006). As previously mentioned homeostasis and budding are tightly linked in Hydra and the first consequence of this autophagy phenotype was to prevent budding and then to slowly induce animal death. These data indicate that the level of autophagy needs to be tightly controlled in steady-state homeostasis.

Interestingly, a deficit of Kazal-type protein activity also leads to excessive autophagy in mammals. In the SpinK$^{−/−}$ newbons, autophagosomes rapidly invades the exocrine pancreatic cells and the surrounding digestive cells (Ohmuraya et al. 2005). As in Hydra, these mice never gain weight and die in a couple of weeks. Therefore, in mice as in Hydra the SpinK3 and Kazal1 proteins that are produced by similar exocrine cells, i.e. the zymogen pancreatic cells and the gland cells respectively, appear to play a similar function, i.e. protect the cells that produce the digestive enzymes and the digestive cells from self-digestion. This is the first example where a similar pathological cellular process regulated by related gene families can be traced from cnidarians to mammals.

Autophagy in eukaryotic cells involves the sequestration and degradation of cytoplasmic organelles via the lysosomal pathway, as such it participates in the maintenance of cellular homeostasis by generating nutrients but also by preventing the accumulation of damaged proteins and organelles (Mizushima et al. 2008). Autophagy, as a vector of diet-induced modulations of homeostasis, is an evolutionarily-conserved mechanism using a highly conserved genetic circuitry. Beside maintaining metabolism, autophagy can also lead to cell death, a mechanism that is widely used across evolution in morphogenetic processes (Melendez and Neufeld 2008). In Hydra where the molecular components of the autophagy machinery are highly conserved, these studies indeed show that two distinct forms of autophagy can be activated, one physiological,
observed during starvation (Buzgariu et al. 2008; Chera et al. 2009a) and a second, pathological, when some regulatory components are deficient (Chera et al. 2006; Galliot 2006).

3. Tissue plasticity in regeneration

One striking aspect of regeneration is its evolutionary distribution in the animal kingdom: the ability to anatomically and functionally restore the lost body parts is widely, but non-uniformly spread in the animal kingdom (Figure 2); also the efficiency and the regenerative strategies used vary a lot not only between different phyla, but even between species of a given phylum (Sanchez Alvarado and Tsonis 2006). The current consensus view is that regeneration was quite common in early animal evolution but submitted to repeated loss or variations during evolution (Sanchez Alvarado 2000; Brookes and Kumar 2008), possibly reflecting ecological constraints (Bely and Nyberg 2010). If true, then it is of utmost interest to decipher the common themes, i.e. the core cellular and molecular processes underlying regeneration in vertebrates as well as in invertebrates (Galliot et al. 2008). We will discuss here several aspects of cellular plasticity that can impact on regeneration but we will leave out one major aspect of regeneration, which is the regulation and function of cell migration towards the wound.

Wound healing in regenerative and non-regenerative contexts

The recovery of tissue integrity in response to environmental stress, injuries or diseases, reunited under the names of *tissue repair* and *regeneration*, requires in multicellular organisms the activation of the wound healing process. Wound healing plays a decisive role in survival by rapidly covering the wound with an epithelial layer that secures body integrity and avoids tissue loss and infections. However in mammals, ageing dramatically affects wound healing: embryos and fetuses are able to heal rapidly, efficiently and without scarring whereas in adults wound healing is often imperfect as in the case of the skin where it is limited to scarring (Redd et al. 2004). Indeed scarring and wound healing are not identical as that latter process requires the unsilencing of repair genes through epigenetic reprogramming in the wound epidermis (Shaw and Martin 2009). An additional level of complexity was observed in vertebrates that regenerate appendages as the wound epidermis that covers the amputation plane thickens to form a structure named Apical Epithelial Cap (AEC), which is an equivalent of the Apical Epidermal Ridge during limb development (Christensen and Tassava 2000; Nye et al. 2003a). This structure delivers signals necessary for the formation and the maintenance of the blastema (Thornton 1957; Nye et al. 2003b). In *Hydra* very little is known about the role played by the stretched ectodermal cells that rapidly cover the wound but pharmacological and RNAi experiments proved that head regeneration does not proceed when wound healing is deficient (Chera, unpublished). In planarians the wound epidermis fulfills a signaling function and few candidate genes were identified in a systematic RNAi screen (Reddien et al. 2005a). Hence it might be possible to trace back some conserved properties of the wound epidermis, possibly lost or deficient in species unable to repair or to regenerate.

Blastema formation, an adult developmental process

*Morphallaxis versus epimorphosis, a sterile debate*

Even though the outcome of regeneration is similar between species, i.e. the *de novo* replacement of the organ or the missing body part, the mechanisms deployed for accomplishing this can be quite different among species and it was so far impossible to outline a unifying view of the cellular and molecular regeneration traits. However the comparative analysis of regenerative contexts showed that the ability of an organism to regenerate depends on its capacity to access a source of pluripotent cells and/or to reprogram differentiated cells (Brookes and Kumar 2002; Odellberg 2005; Poss, 2007; Birnbaum and Sanchez Alvarado 2008). These cells then adopt a regeneration-specific behavior that was heavily investigated in amphibians, fish, insects and planarians. We will briefly review the general concepts that emerged from these studies.

The regenerative strategies that lead to the rebuilding of complex, multi-tissue structures are classically considered as either *morphallactic*, i.e. proceeding through re-patterning the pre-existing tissue in the absence of cell proliferation, or *epimorphic* i.e. relying on the proliferation of undifferentiated progenitors that form a regeneration-specific structure, named the blastema (Morgan 1901). This transient mass of mesenchymal proliferating cells is a self-organizing structure, which upon transplantation keeps the memory of its origin and drives patterning, differentiation and morphogenesis of the regenrated structure (Stocum 1968b; Stocum 1968a). As a general rule the blastema, which requires the AEC and some neurotrophic factors for its growth (Tassava and Garling 1979; Kumar et al. 2007), senses the discontinuity with the remaining structure named stump. This sensing promotes the setting up of the proximal and distal boundaries and their confrontation leads to the regeneration of intermediate structures that intercalate until discontinuity is filled (Nye et al. 2003b). These principles that were uncovered thanks to transplantation experiments of amphibian limb blastemas (Iten and Bryant 1975; Stocum 1975), were also identified in insects that regenerate their appendages (French et al. 1976; Nakamura et al. 2008), suggesting that they were already at work in the last common bilateral ancestor.

However, these two regenerative strategies, morphallactic versus epimorphic, might well be two extreme poles of a continuum that would better represent the variable complexity of the multiple distinct regenerative contexts. For example, if the nerve supply is deficient, i.e. the blastema does not
grow properly, but in the presence of the AEC, a miniature limb can regenerate, reminiscent of a morphallactic process (Nye et al. 2003b). Also in planarians, blastema formation results from a mixed morphallactic-epimorphic process that varies according to the site of the amputation (Salo and Baguna 1984; Agata et al. 2007). Therefore opposing morphallaxis and epimorphism actually promotes a rather reductionist view of regeneration, which prevents its understanding (Agata et al. 2007).

Following this vein, the question of the role of stem cells and proliferating cells in Hydra regeneration was recently revisited. Classically Hydra regeneration is considered as morphallactic, with two types of arguments supporting this statement, first the absence of epithelial cell proliferation in head regenerating halves, at least on the first day following bisection (Holstein et al. 1991), and second the fact that animals exposed to anti-mitotic drugs still regenerate their head (see in Bosch 2007). However in wild-type Hydra interstitial progenitors and interstitial stem cells rapidly divide in head regenerating stumps after mid-gastric bisection but not after decapitation, forming a blastema-like structure that drives head regeneration (Chera et al. 2003b). These results indicate that regeneration in Hydra is more plastic than anticipated, following distinct routes when the amputation level varies: At mid-gastric position head regeneration displays some features of epimorphic regeneration, whereas after decapitation it is morphallactic. The cellular backgrounds are indeed quite different at these two positions; at mid-gastric position, a large number of cells are stem cells whereas in the upper body column, cells are already committed to a given pathway or on the way to differentiate (Steele 2002). Thus the cellular contexts at the time of injury dramatically influence the regenerative route taken immediately after injury (Figure 5).

**How to induce and grow a blastema?**

Two main distinct strategies to form a blastema were identified: a direct one relying on the **recruitment of stem cells**, and another more indirect relying on **cell plasticity**, i.e. on the **dedifferentiation of adult cells** located in the vicinity of the wound (Figure 5). Planarians illustrate the first case where the formation of blastema requires the recruitment of adult stem cells named neoblasts (Reddien and Sanchez Alvarado 2004). Similarly, *Xenopus* tadpoles that regenerate their tail do not use dedifferentiation but rather recruit satellite cells, which are small Pax7+ stem cells located in the basement membrane of the skeletal muscle (Slack et al. 2008). Recently, these satellite cells were also proposed to participate in blastema formation in amputated salamander limbs (Morrison et al. 2006). However a large number of studies indicate that blastema formation in urodeles regenerating their appendages is predominantly indirect, relying on the dedifferentiation of numerous cell types (multinucleated myocytes, fibroblasts chondrocytes, Schwann cells) into cycling progenitors (Hay 1959; Geraudie and Singer 1981; Muneoka et al. 1986; Lo et al. 1993). The pluripotency of these progenitors was often assumed but never proven (for reviews see Brookes and Kumar 2002; Bryant et al. 2002; Echeverri and Tanaka 2002).

Recently the Tanaka’s group performed systematic cell lineage tracing studies in transgenic animals, and showed that the plasticity of these progenitors is actually more restricted than anticipated: they keep the memory of their cellular origin and re-differentiate in the regenerated structure according to this origin (Kragl et al. 2009). These direct and indirect mechanisms of blastema formation are not mutually exclusive and can be combined in different proportions in distinct tissues of the same regenerative animal (Figure 5). Also the dominant process might not be exclusive: it was recently proposed that neoblasts surviving non-lethal irradiation might result from the dedifferentiation of radio-resistant differentiated cells (Salvetti et al. 2009). If confirmed, this suggests that cellular events that are rare and thus difficult to detect, might actually become transiently accessible in restricted regeneration conditions. In *Hydra* evidences for cell dedifferentiation are lacking.

**Transdifferentiation, a special case of injury-induced plasticity**

Some regenerative processes rely on transdifferentiation of differentiated cells (as defined above) rather than on recruitment of stem cells or progenitor cells for blastema growth. In homeostatic adult tissues, transdifferentiation is a rare event, but its frequency increases upon injury. One of the best-defined examples of organ regeneration through transdifferentiation in vertebrates is the induction of lens from the pigmented epithelium of the newt iris. This process, named Wolffian lens regeneration, was identified in newt, fishes and chick embryo by the group of Goro Eguchi who could observe it *in vivo* and reproduce it *in vitro* (Eguchi and Kodama 1993). *In vivo* the cells of the dorsal iris (but not the ventral one) are competent, activating the Six3 transcription factor to regenerate the lens after lentectomy (Grogg et al. 2006). More generally in vertebrates, spontaneous transdifferentiation appears in contexts involving organ regeneration, such as lens, retina, liver, pancreas (Slack 2007).

In *Hydra* transdifferentiation appears common, as exemplified by the ganglia neurons that undergo at the time they get displaced along the oral-aboral axis a phenotype conversion (Bode 1992). This phenotype conversion was identified thanks to nerve-specific epitopes expressed in subsets of ganglia neurons at the extremities but not in the body column. Surprisingly animals totally depleted in neuronal progenitors after nitrogen mustard or hydroxyurea treatments, can re-express some of these markers in apical neurons after decapitation, thus most likely arising in differentiated neurons that were not expressing them before bisection (Koizumi and Bode 1986; Yaross et al. 1986). However this phenotype conversion does not fulfill the criteria of transdifferentiation as changes in cellular morphology were not identified.
A more striking example of transdifferentiation in *Hydra* is that of ganglia neurons of the body column that after bisection become epidermal sensory nerve cells in the regenerated structure, head or foot (Koizumi et al. 1988; Koizumi and Bode 1991). Such conversions require the differentiation of a new anatomical structure, the cilium, which is missing in ganglia neurons. More recently, the specific expression of GFP in gland cells of transgenic *Hydra* was used to trace a similar transdifferentiation event: During head regeneration after decapitation, the zymogen gland cells of the body column are converted to mucous gland cells in the *de novo* formed head region (Siebert et al. 2008). Nevertheless these data do not tell us whether transdifferentiation is a by-product of injury or a major player in regenerating *Hydra*. For example, does a head properly regenerate when transdifferentiation is inhibited? To address such question, the combination of cell lineage tracing in transgenic animals to RNAi loss of function assays should help evaluate the contribution of transdifferentiation to *Hydra* regeneration. By contrast in the jellyfish *Podocoryne* (a species closely related to *Hydra*) transdifferentiation appears as a driving force for regeneration: striated muscle cells can be induced to differentiate to smooth muscle cells as well as neurons by disrupting the interactions between cells and the extra-cellular matrix (Schmid and Alder 1984; Schmid and Reber-Muller 1995). This induced transdifferentiation event requires cell proliferation and is able to support regeneration of the feeding organ (the manubrium).

In planarians, transdifferentiation of terminally differentiated cells is poorly documented but the plasticity of post-mitotic cells in the blastema was established: these cells that are already fate-committed can modify their fate according to the surrounding tissue (Newmark et al. 2008). More surprisingly, cytophotometric and karyological analyses have shown that germ cells can be recruited into the blastema to adopt a somatic cell fate after injury (Gremigni et al. 1980b; Gremigni et al. 1980a). These examples of cellular plasticity in planarians rather correspond to transdetermination than transdifferentiation events.

In conclusion, tissue restoration relying on transdifferentiation represents a case where a form of cell plasticity that is rare and unusual in homeostatic conditions is triggered and enhanced by the injury stimulus. As such it provides interesting possibilities for regenerative medicine. However the molecular mechanisms underlying transdifferentiation-driven regeneration are still poorly understood. Planarians and *Hydra* certainly provide fully appropriate systems to investigate how and when transdifferentiation contributes to regeneration.

4. Origin(s) of Regeneration

Regeneration, the other face of asexual development?

The fact that the regenerative abilities are strongest in species that can propagate asexually, like budding in *Hydra*, fission in planarians or annelids (Sanchez Alvarado 2000; Brockes and Kumar 2008; Bely and Nyberg 2009) suggest that regeneration and asexual reproduction might share some evolutionary history. One possible scenario would be that regeneration evolved from asexual reproductive mechanisms, conferring some adaptive advantages that might have sustained its perpetuation across evolution. Considering this view, Candia-Carnevali proposed to consider “regeneration as the necessary and complementary developmental process associated with asexual reproduction, in analogy with embryogenesis as being the developmental strategy complementary to sexual reproduction” (Candia-Carnevali 2006). The reproductive and regenerative properties of different protozoans, which generate two new complete individuals by fission or splitting, favor this hypothesis of a common origin for asexual reproduction and regeneration. The main difference between asexual reproduction and regeneration in protozoans as well as in *Hydra* appears to be the stimulus triggering these two events: a favorable environmental conditions such as abundance of food in the first case, a deleterious incident, such as injury in the latter one. In annelids, similar gene expression patterns recorded during both fission and regeneration were interpreted as an evidence of a shared genetic circuitry (Bely and Wray 2001).

However several arguments challenge this proposal. First regeneration also takes place in numerous species that do not display asexual reproduction, such as urodeles, teleost fish. But of course each of these two processes likely had its own evolutionary history and the loss of asexual reproduction across evolution might have occurred multiple times without affecting regenerative processes (Bely and Nyberg 2010). Second although in *Hydra* the developmental programs that takes place when the head forms, appear highly similar during regeneration, budding and sexual development (Gauchat et al. 1998; Technau and Bode 1999), distinct signaling pathways appear to regulate initiation of budding and initiation of regeneration in *Hydra* (Fabila et al. 2002; Chera et al. unpublished). And indeed only budding and not regeneration is inhibited in starved *Hydra* (Figure 4). Therefore we assume the early signaling that links injury to the reactivation of head formation might be regeneration-specific. According to that scenario, regeneration and asexual reproduction would converge on the same developmental program, here to form a new head, but would differ by the module that activates it.
Regeneration, a continuum of development?

What is an adult organism?
For long regeneration was considered as a developmental process that takes place during adulthood but still tightly bound to organogenesis and to a lesser extent to embryogenesis. This view led to the hypothesis according to which regeneration results from the reactivation of larval/fetal (possibly embryonic) developmental processes in adulthood. However this strict definition certainly does not cover the regeneration field: Firstly regenerative processes also take place in non-adult organisms at various periods of their life cycle, and secondly adulthood, which is defined by the acquisition of sexual maturity, is an ambiguous concept. In fact the level of adulthood (what we propose to name adulthoodness) at the time regeneration is initiated in an organism is highly variable: It varies between species as some species show the persistence of juvenile traits in adulthood, and it varies between individuals of a given species as ageing obviously affects the regenerative potential. Therefore “adulthoodness” should also be considered as an important parameter to compare the different regenerative contexts and understand the principles of regeneration, as more adulthoodness likely means less developmental activity and vice-versa. To take into account these two parameters, life cycle stage and adulthoodness, we sorted a series of regenerative contexts according to the developmental/adult status of the organism at the time of injury (Table 1).

Sorting out of the regenerative processes according to developmental criteria
Regeneration of type 1 includes all “regulative processes” that take place in the embryonic period as the half of the Xenopus embryo that regenerates a complete embryo (Reversade and De Robertis 2005) or the chick embryo regenerating its neural tube (Ferretti and Whalley 2008). As discussed above (see developmental plasticity), these cannot be considered sensu stricto as regenerative processes as the developmental program is broadly active at the time the structure is amputated and re-built. Regeneration of type 2 corresponds to “fetal/larval regeneration”, it occurs during organogenesis or larval stages but extincts after metamorphosis or birth. Typical examples are insect larvae (the cricket nymph, the Drosophila) that regenerate their appendages (McClure and Schubiger 2007; Nakamura et al. 2008) or the Xenopus tadpole that regenerates its tail (Slack et al. 2008). After metamorphosis or birth, the ageing dimension should be taken into consideration as in most species juvenile organisms certainly show a stronger regenerative potential than the sexually mature or aged individuals from the same species. Therefore we consider “juvenile regeneration” as a separate type (type 3), taking place in fully developed organisms before they reach sexual maturity.

Type 4 regeneration is named “paedomorphic” and likely shares similarities with types 2 and 3 as it takes place in sexually mature organisms that are characterized by the persistence of juvenile traits at adulthood. In such species the developmental timing is shifted when compared to closely related species: either the sexual development takes place too early (progenesis) or the somatic development is slowed down and not achieved at the time sexual maturity is established (neoteny). Consequently these animals reach sexual maturity without displaying the full panel of somatic ancestral features of adulthood (Gould 2000). There are two good examples of paedomorphic species with high regenerative potential: the Hydra polyps that are sexually mature as polyps and not as medusa, which is the ancestral adult status in medusozoans (Hydra actually lost the medusa stage) and the neotenic Axolotl salamander that does not undergo metamorphosis, remaining aquatic instead of terrestrial all along its life. The high regenerative potential of Axolotl reflects an easier access to developmental programs despite the sexually mature status; this potential was actually proposed to be secondarily acquired (Tanaka and Ferretti 2009). Finally a 5th type is “adult regeneration” as it takes place in animals that reached both sexual and somatic maturity. In fact adult regeneration is highly variable, either complete as in planarians, or quite broad as in teleost fish and urodeles that regenerate appendages, nervous system, organs, or restricted to tissue repair as in most mammals.

The developmental part of the regeneration program
The correlation between the strength of the regeneration potential and the developmental status indeed indicates that when the developmental programs close, the regenerative potential is drastically altered in most species. Therefore the analysis of regeneration in developing organisms offers the possibility to understand how this property is silenced during development, at the time of metamorphosis or when sexual maturity is established. If the “development continuum” hypothesis holds true then the genetic circuits supporting development and regeneration should be highly similar if not identical. Indeed the regenerative re-patterning mechanisms often deploy developmental genes. In urodeles (paedomorphic or adult regeneration) as in the Xenopus tadpole (larval regeneration) the genetic programs supporting limb development and limb regeneration share similarities, although restricted to the last phase of the developmental and regenerative processes, at the time the limb bud forms and grows (Bryant et al. 2002; Pearl et al. 2008). Similar conclusion was obtained in Xenopus tadpoles after induction of tail regeneration at the refractory period (Beck et al. 2003).

Hence, one of the questions currently under investigation is whether regeneration partially reiterates embryogenesis and/or organogenesis, following generic-patterning mechanisms, or alternatively makes use of regeneration-specific circuitries that include developmental genes (Birnbaum and Sanchez Alvarado 2008; Brockes and Kumar 2008). There is no clear answer to this question as in fact only few regeneration-specific mutants were
characterized (Behra et al. 2009) and regeneration-specific circuits remain to be identified. Obviously adult regeneration contexts (type 5) are most favorable to characterize such circuits.

In contrast to the late phase of regeneration characterized by morphogenesis and growth, the cellular and genetic programs at work at the initial and intermediate phases of regeneration (wound healing and blastema formation respectively) do not match the determination and initiation phases of embryogenesis (Brockes and Kumar 2005). This result is actually not so surprising as regeneration and development occur in distinct contexts and at distinct scales; during regeneration, in response to the tissue loss, the cells reorganize within preexistent tissues in injury-induced stress conditions, whereas during development the cells migrate and differentiate in specific tissues, which form structures following strict temporal-spatial rules. At the molecular level, the injury response recruits the stress response pathways (Pearl et al. 2008) whereas blastema formation relies on signals with no or limited activity during development (Kumar et al. 2007; Yin and Poss 2008). As a conclusion, regeneration certainly does not simply recapitulate development even though it makes use of tools previously used during embryonic and fetal/larval development.

**Regeneration, a continuum of homeostasis?**

The recent tremendous development of the stem cell biology and cell plasticity fields helped take a novel viewpoint, no longer considering regeneration as strictly developmental, but also as a cell biology problem where the dynamics of tissues in steady-state conditions would dramatically influence the response of the organ or the body to stress and injury within a given time frame window (Birnbaum and Sanchez Alvarado, 2008). This clearly provides a different angle to decipher the enigma of the variability in the regenerative potential. In the next sections we will discuss how homeostasis might impact on regeneration.

**Are the mechanisms that maintain homeostasis recruited in response to injury?**

**Autophagy in regeneration**

The *Hydra* model system is well suited to test during regeneration the role of processes that maintain homeostasis. As reported above, autophagy is an essential process to survive long periods of starvation in *Hydra* (Buzgariu et al. 2008; Chera et al. 2009a). When *Hydra* are exposed for 2 hours at the time of bisection to pharmacological drugs that induce or inhibit autophagy, head regeneration is only slightly delayed, suggesting that transient modulations in the level of autophagy do not dramatically affect regeneration. In contrast in *Kazal1* RNAi knocked-down *Hydra* the amputation stress appears to immediately enhance the preexisting level of autophagy in the endodermal layer. Interestingly this immediate post-injury autophagy is reversible in few hours if the pre-injury autophagy is moderate, but no longer reversible when the pre-injury autophagy is high. Therefore high levels of autophagy in the endoderm at the time of bisection are not compatible with the stress of the amputation (Chera et al. 2006; Galliot 2006).

Consequently the mechanisms that limit the level of autophagy after amputation certainly play an essential cytoprotective function; again similar mechanisms apply in *Hydra* and mammals, one of them would be the up-regulation of protease inhibitors in the injured region (Neuschwander-Tetri et al. 2004). These data suggest that two distinct types of autophagy with opposite regulations develop in chronic and acute contexts, a slow and positive one that progressively increases during diet restriction to support tissue survival, a fast and negative one that rapidly leads to cell death after injury if not repressed. It is currently not clear how much is shared between these two types of autophagy. In planarians, a report suggests some role for autophagy during regeneration and starvation (Gonzalez-Estevez et al. 2007).

**Apoptosis in regeneration**

Apoptosis also seems to play quite different roles in homeostasis and regeneration in *Hydra*. The wave of apoptosis that takes place in head-regenerating tips immediately after mid-gastric section, affects about 50% of the cells in the first hour following injury and is critical to induce the proliferation of the surrounding progenitor cells as apoptotic cells release signaling molecules as Wnt3 (Chera et al. 2009b). By contrast less than 1% of the cells are apoptotic in homeostatic conditions (up to 2% during starvation), these cells are distributed along the body column and are supposed to maintain the cell mass in a steady state (Bosch et al., 1984).

Apoptosis is actually emerging as an important process to bridge injury to regeneration: it was observed during planarian regeneration (Hwang et al. 2004; Pellettieri et al. 2009) and is required during the first day of tail regeneration in the Xenopus tadpole (Tseng et al. 2007). In two other contexts, wing discs of *Drosophila* larvae and head regenerating tips in *Hydra*, injury-induced apoptotic cells were actually shown to induce compensatory proliferation by releasing signaling molecules as Wg/Wnt3, Dpp or Hedgehog (Huh et al. 2004; Perez-Garijo et al. 2004; Ryoo et al. 2004; Fan and Bergmann 2008; Chera et al. 2009b).

Two non-mutually mechanisms were proposed as to the role of the apoptotic cells: either the recruitment of stem cells and progenitors via a direct signaling and/or the selective destruction of cells that normally exert a negative pressure on the cycling activity of progenitors and stem cells (Simon et al. 2009). A recent study that investigated the function of apoptotic cells in mice, showed that indeed injury-induced cell death efficiently triggers cell proliferation in vitro and in vivo, as well as tissue repair in skin and liver (Li et al. 2010). These authors identified the activated caspases 3 and 7 as key players to generate arachidonic acid, itself converted into prostaglandins that stimulate
proliferation of stem cells; they named this mode of signaling the "phoenix rising" pathway. As apoptosis by itself is an extremely fast process (about 1 hour), its role was likely overlooked in the past but recent tools as apoptosis sensors should soon help identify the regulation(s) and action(s) of this pathway across various regenerative contexts.

**Adult stem cells as a direct support to build a regenerative response**
A determining factor to potentiate tissue repair and regeneration is the intensity of self-renewal in homeostasis. The direct influence of the ASCs on the regenerative response to injury or stress support the "homeostasis continuum" hypothesis and open new hopes for establishing a regenerative medicine. Hydra and planarians provide robust experimental model systems to decipher how the biology of ASCs impact on the regeneration potential.

**ASCs in the Hydra and planarian regenerative responses**
*Hydra* regeneration requires complex and variable interactions between the epithelial and interstitial stem cells. As a fruitful experimental approach, interstitial stem cells can easily be eliminated either after a short antimitotic treatment or after heat-shock in the sf-1 temperature-sensitive mutant, producing "epithelial" animals unable to catch their food but able to bud and regenerate although with less efficiency (Campbell 1976; Marcum et al. 1980). This proves that epithelial stem cells can drive morphogenesis in the absence of interstitial stem cells. Indeed epithelial cells were shown to produce signaling molecules and epitheliopeptides involved in morphogenesis (Fujisawa 2003; Guder et al. 2006; Lengfeld et al. 2009). However the interstitial stem cells also likely play a role in this flexible scenario; they produce signaling peptides (Schaller et al. 1989), they interact with epithelial cells to finely tune their morphogenetic potential (Sugiyama and Wanek 1993), they appear essential to trigger the head regeneration program after mid-gastric section (Chera et al. 2009b), they participate in head homeostasis and head regeneration by regulating apical neurogenesis (Miljkovic-Licina et al. 2007). Transplanting interstitial stem cells into epithelial Hydra demonstrated their multipotency and the plasticity of nerve precursors (Holstein and David 1990b; Fujisawa 1992; Minobe et al. 1995). Similarly experiments combined to BrdU labeling identified populations of interstitial cells cycling at distinct paces (Holstein and David 1990a). Therefore future studies should tell us more about the plasticity of the epithelial and interstitial stem cells and about the way they interact to form stem cell niches that contribute to regeneration in *Hydra* (Galliot et al. 2006; Wittlieb et al. 2006; Bosch 2009).

In planarians the rapid proliferation of neoblasts is essential to mount the regenerative response; their role was recognized since decades thanks to irradiation experiments that eliminate them (Wolff and Dubois 1948; Salo and Baguna 1985) and more recently to BrdU-labeling experiments that proved that neoblasts are indeed the only cells to divide in planarians (Newmark and Sanchez Alvarado 2000). Neoblasts form in fact a heterogeneous cell population (Reddien et al. 2005b; Hayashi et al. 2006) and use evolutionarily-conserved genetic programs to regulate stemness (Reddien et al. 2005b; Guo et al. 2006; Rossi et al. 2007; Eisenhoffer et al. 2008). As an example, the PTEN/TOR pathway, a critical regulator of self-renewal of stem cells (Hill and Wu 2009), is required for regeneration. In PTEN RNAi knocked-down planarians, a pseudo-metastatic process was observed with neoblast proliferation, disorganization of differentiated tissues and loss of basal membrane integrity, indicating that the biology of stem cells is dramatically impaired (Oviedo et al. 2008). In *Hydra* the PTEN/TOR pathway is present (Chera et al. 2009a) but its function was not tested yet. Further comparative analyses should tell us more about the regulatory pathways shared between *Hydra* stem cells and planarian neoblasts.

**Adult neurogenesis from cnidarians to mammals**
One tissue that was thought for long to be refractory to tissue repair is the central nervous system (CNS), which once developed would not be able to self-renew. However over the recent years adult neurogenesis was identified in mammals, including humans, in two regions of the CNS, the hypothalamus and the olfactory bulb (Suh et al. 2009). The plasticity of the neural stem cells identified in these two locations might provide a source for regenerative medicine. In amphibians, reptiles and teleost fish adult neurogenesis is even more widely distributed (Kaslin et al. 2008; Zupanc 2008), associated in some species to the regeneration of the CNS (Tanaka and Ferretti 2009). Also urochordates as *Ciona* exhibit a strong but age-dependent capacity for regenerating their central nervous system (Auger et al. 2010; Dahlberg et al. 2009). Finally in *Hydra*, all cell types of the adult nervous system (sensory cells, ganglia cells, mechano-receptor cells) are continuously replaced in homeostatic conditions and the nervous system that is denser and organized at the apical pole is fully regenerated in few days after amputation (Bode 1996; Galliot et al. 2009).

The molecular and cellular basis of this plasticity is not known yet but some candidates were already identified. For example the maintenance of adult apical neurogenesis and the *de novo* neurogenesis during head regeneration are dramatically impaired when the Gsx paraHox transcription factor, which is expressed in neuronal progenitors and apical neurons, is RNAi knocked-down (Miljkovic-Licina et al. 2007). Interestingly this transcription factor is also involved in neurogenesis in developing mice, specifying the identity of a subset of telencephalic progenitors during development (Yun et al. 2003). It would be of interest to know whether Gsx orthologs also play a role in vertebrate adult neurogenesis. Similarly, the CREB transcription factor appears as a key regulator of neurogenesis from *Hydra* (Chera et al. 2007) to zebrafish and mice (Dworkin et al. 2007; Dworkin et al. 2009).
In addition some of these model systems as the zebrafish allow to compare developmental and adult neurogenesis (Zupanc 2008) and to characterize the stem cell niches where neuronal progenitors keep proliferating in adulthood (Kaslin et al. 2009). This is of utmost importance as recent studies indeed revealed clear differences between embryonic and adult stem cells, likely reflecting the age-dependent variations of the mechanisms supporting stem cell function (Levi and Morrison 2008; Suh et al. 2009). In short self-renewal becomes deficient over time, impacting on the size of the stem cells stock and thus reducing the regeneration potential. *Hydra oligactis*, a species where ageing is observed when sexual differentiation is induced (Yoshida et al. 2006) provides a suitable experimental framework to test the mechanisms that link ageing process, adult neurogenesis and regeneration of the nervous system.

5. A model of modular organization for regeneration

Reviewing the various aspects of plasticity, we have inspected many pieces of the homeostasis and regeneration puzzles. In this last section, we will try to see how these pieces might attach together to mount a regenerative response, obeying some rules that might apply from *Hydra* to vertebrates.

The early phase of regeneration draws its tools from homeostatic plasticity

The cellular properties of the immediate and early phases of regeneration that are distinct from embryogenesis or organogenesis by a number of criteria (Brockes and Kumar 2005), seem to share more with the dynamics of homeostasis. Typically only organisms, organs or tissues that maintain a dynamic homeostasis based on cell renewal or cell plasticity, are able to launch a regenerative response after injury. However this injury-dependent regenerative response takes place neither at the same scale nor at the same speed when compared to homeostasis: processes that are extremely rare and difficult to observed in homeostatic conditions occur within a short period of time after injury, often affecting a much larger number of cells. For example injury promotes the conditions for generating a regenerative response by dramatically enhancing the level of apoptosis, by promoting transdifferentiation, by inducing dedifferentiation, or by pushing stem cells, progenitor cells or even differentiated cells to cycle. These two criteria, the speed and the magnitude of the injury-induced cellular processes, appear as a common theme between the various regenerative contexts rather than the processes themselves. Finally some aspects of regeneration as positional memory, nerve dependence of the blastema growth appear neither as a continuum of homeostasis, nor as a continuum of development, but fully specific to regeneration (Kumar et al. 2007). Whether they obey to common rules in vertebrate and invertebrate contexts is currently not known.

The late phase of regeneration mimics development

As discussed above, the correlation between asexual reproduction and regeneration in a number of species (cnidarians, planarians, annelids) might reflect a common origin for these two processes. This question can be addressed in *Hydra* where head formation can easily be compared in three distinct adult developmental contexts, budding, head regeneration after decapitation, head regeneration after mid-gastric section. Preliminary results indicate that asexual reproduction and head regeneration follow a similar structure, formed of successive modules. The last module that we name “development-like module”, supports the formation of the new head and appears highly similar if not identical between asexual reproduction and the two types of regeneration. Moreover these adult developmental programs seem to closely resemble the embryonic/fetal programs involved in head formation (Technau and Bode 1999). Therefore as in vertebrates, the late phase of regeneration, i.e. the differentiation of the *de novo* structure appears to re-use with similar rules the tools previously used during development. If confirmed, this
conservation indicates that the development-like module of a specific structure remained highly constrained across evolution.

**Regeneration results from the sequential activation of homeostasis-derived and development-like modules**

By contrast the mechanisms that lead to the activation of the developmental module seems to be multiple. As they all precede the differentiation of the de novo structure, we have grouped them into the "induction module". In Hydra the induction module corresponds to the induction of budding, i.e. the formation of the bud spot on the parental polyp, or the activation of the regeneration program through the injury responses (Figure 5). As we saw, these injury responses are actually quite different after decapitation or after mid-gastric bisection (morphallactic or epitomorph-like respectively). Therefore we suspect that the variability of the induction module actually directly reflects the parameters that define each component of the homeostatic plasticity within a given tissue, a given organ (transdifferentiation, dedifferentiation, recruitment of stem cells, proliferation of differentiated cells...). These components constrain the regenerative plasticity that will be developed upon injury.

Finally the immediate module is the wound healing response (of course absent when injury or stress are lacking as during budding). Its major regenerative function, besides preserving tissue, organ, structure integrity, would be to rapidly amplify the components of homeostatic plasticity to convert them to regenerative plasticity as defined by the induction module. Whether the impact of wound healing on the activation of the induction module is fixed or plastic is unknown. This modular organization of the regenerative process resulting from a combination of highly constrained development-like modules that are structure-specific and much more variable homeostatic-dependent induction modules can account for the diversity of the regenerative responses, sometimes even in the same organism as observed in Hydra regenerating its head. The level of adulthood presumably strongly modulates the accessibility to the development module but can also impact on the parameters of the induction module. In the context of tissue repair, the developmental module would be either not available or truncated, as a consequence the wound healing and the induction module would suffice to replace the missing tissue but not to regenerate a pre-existing three-dimension structure.

**6. To conclude**

Homeostasis and regenerative processes rely on the coordinated integration at the tissue level of multiple forms of cellular plasticity. Our most recent knowledge in stem cell biology indicates that the molecular changes that support cellular and developmental plasticity rely on epigenetic nuclear modifications.

The Hydra and planarian model systems possess unique features to study stem cell biology, maintenance of homeostasis and reactivation of developmental programs in adulthood. Bisected Hydra polyps allow study tissue repair (foot regeneration) as well as different routes to achieve a complex form of regeneration, i.e. head regeneration.

In Hydra some sustained cellular adaptations required to maintain homeostasis, i.e. a massive autophagy and a moderate apoptosis, do not exhibit similar regulations during the regenerative response. By contrast a limitation of autophagy is required at the tip to promote cell survival after amputation and a wave of apoptosis that induces proliferation of the surrounding progenitors is needed in head-regenerating tips after mid-gastric bisection.

We propose to view animal regeneration as an adult developmental process with a tripartite modular organization: the wound healing response, the regeneration induction module and the developmental module. The wound healing response amplifies the various forms of plasticity available prior to injury, the regeneration induction module develops a cellular remodeling that integrates these different forms of plasticity to activate the developmental module, which appears to make use of the tools previously used during embryogenesis or organogenesis.

The regeneration induction module, highly constrained by the homeostatic conditions, allows to bridge the wound healing response to the reactivation of a developmental program by using one or several forms of cellular plasticity that can be combined: proliferation of differentiated cells, dedifferentiation and proliferation of precursor cells, stem cell recruitment, apoptosis-induced compensatory proliferation, transdifferentiation. In numerous species, but not in mammals, the regeneration induction module corresponds to the formation of the blastema. In amphibians the dedifferentiation of adult somatic cells after injury does not seem to lead to pluripotency in the blastema.

Five distinct forms of regeneration can be identified according to the developmental status of the regenerating organism: regulative, fetal-larval, juvenile, paedomorphic, adult. We assume that the importance of the regeneration induction module inversely correlates with the intensity of the developmental processes ongoing at the time of injury.

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