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Non-developmental dimensions of adult regeneration in *Hydra*

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ABSTRACT An essential dimension of 3D regeneration in adult animals is developmental, with the formation of organizers from somatic tissues. These organizers produce signals that recruit surrounding cells and drive the restoration of the missing structures (organs, appendages, body parts). However, even in animals with a high regenerative potential, this developmental potential is not sufficient to achieve regeneration as homeostatic conditions at the time of injury need to be "pro-regenerative". In *Hydra,* we identified four distinct homeostatic properties that provide a pro-regenerative framework and we discuss here how these non-developmental properties impact regeneration. First, both the epithelial and the interstitial-derived cells are highly plastic along the animal body, a plasticity that offers several routes to achieve regeneration. Second, the abundant stocks of continuously self-renewing adult stem cells form a constitutive pro-blastema in the central body column, readily activated upon bisection. Third, the autophagy machinery in epithelial cells guarantees a high level of fitness and adaptation to detrimental environmental conditions, as evidenced by the loss of regeneration in animals where autophagy is dysfunctional. Fourth, the extracellular matrix, named mesoglea in *Hydra,* provides a dynamically-patterned environment where the molecular and mechanical signals induced by injury get translated into a regenerative process. We claim that these homeostatic pro-regenerative features contribute to define the high regenerative potential of adult *Hydra*.

KEY WORDS: *Hydra regeneration, adult stem cell, autophagy, plasticity of epithelial stem cell, homeostatic pro-blastema*

Introduction

Hydrozoan polyps regenerate their body by forming organizers

The discovery that some animals are able to fully regenerate their body was initially made on freshwater *Hydra* polyps by Abraham Trembley (Trembley, 1744). Since then, *Hydra* remained a fruitful experimental model system to study regeneration (Holstein *et al.*, 2003; Bosch, 2007; Galliot, 2012) (Fig. 1). As an extreme case of regeneration, cell suspensions obtained from dissociated healthy *Hydra* tissues can regenerate into complete animals, a process named reaggregation, which demonstrates that no pre-existing tissue polarity is required to form a new animal (Gierer *et al.*, 1972). These amazing properties are quite common in hydrozoans, the class of Cnidaria to which *Hydra* belongs. Hydrozoans are most often marine animals that exhibit a complex life cycle, with embryos developing as swimming larvae (planulae), which undergo metamorphosis into sessile hydroids (polyps, most often colonial), until polyps bud swimming jellyfish that are able to sexually reproduce (medusae). Although *Hydra* is highly derived among hydrozoans (it lives exclusively in freshwater and its life cycle is reduced to a solitary polyp stage, i.e. no planula nor medusa stages), its amazing regenerative potential should not be considered as a zoological anomaly, but rather as a property shared between hydrozoan polyps, such as *Corymorpha, Tubularia, Hydractinia* (Tardent, 1963; Bradshaw *et al.*, 2015).

Indeed, the study of these animals contributed and continues

Abbreviations used in this paper: ASC, adult stem cell; dsRNA, doublestranded RNA; ECM, extra-cellular matrix; ESC, epithelial stem cell; eESC, epidermal epithelial stem cell; gESC, gastrodermal epithelial stem cell; *Ho_CR, Hydra oligactis* cold-resistant; *Ho_CS, Hydra oligactis* cold-sensitive; *Hv, Hydra vulgaris*; ISC, interstitial stem cell; ROS, reactive oxygen species.

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to contribute to the dissection of common principles of animal regeneration, as well as to the identification of pro-regenerative conditions. A main breakthrough in the understanding of the mechanisms of regeneration was the identification and the characterization in Hydra of organizers, i.e. tissues that produce signals to recruit surrounding cells and drive the development of the appropriate missing structure (Browne, 1909; Yao, 1945; Webster and Wolpert, 1966; Broun and Bode, 2002). Formation of organizers, also at work during developmental processes of vertebrates (Joubin and Stern, 2001), is essential for regenerative processes that involve 3D reconstruction across evolution (Pfefferli and Jazwinska, 2015; Vogg et al., 2016). Two main signaling systems appear to promote organizer formation in Hydra regenerating its head: the Wnt/b-catenin signaling pathway (Hobmayer et al., 2000; Chera et al., 2009b; Lengfeld et al., 2009; Nakamura et al., 2011; Vogg et al., 2018) and the BMP/chordin/noggin pathway (Reinhardt et al., 2004; Rentzsch et al., 2007; Chandramore et al., 2010). However the conditions that promote the transition from wound healing immediately after bisection to organizer formation is not well understood.

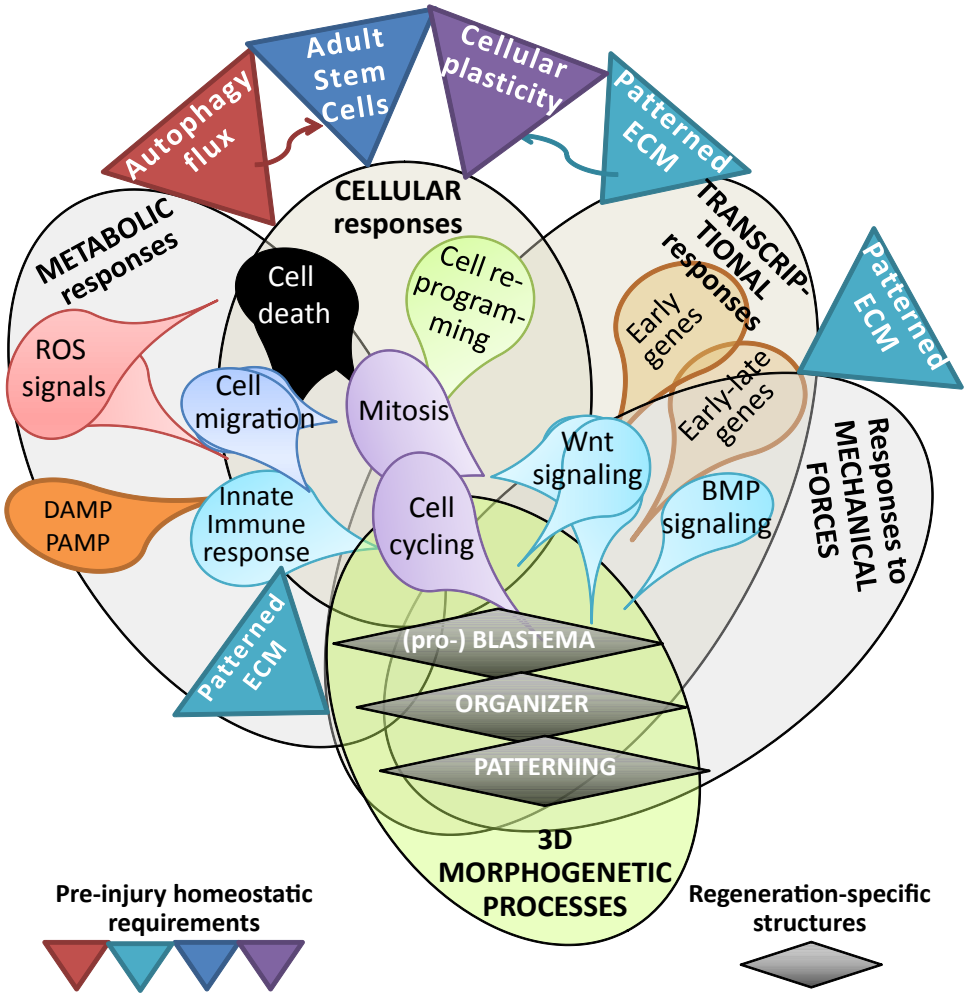

Fig. 1. The developmental and non-developmental dimensions of adult regeneration in *Hydra*. *The non-developmental dimensions relevant for adult regeneration are those identified for body regeneration in* Hydra*, which are four: a dynamically-patterned extra-cellular matrix (ECM), abundant stocks of continuously self-renewing adult stem cells, a high level of cellular plasticity in both epithelial and interstitial-derived cells, and a tightly-tuned autophagy flux. See references in the main text.*

Large stocks of Adult Stem Cells populate the Hydra body column

In most bilaterian contexts, regeneration requires the formation of a proliferative blastema, and the limited potential for regeneration in mammals is interpreted as the result of their inability to induce blastema formation after amputation (Tanaka and Reddien, 2011). In *Hydra*, large stocks of cycling Adult Stem Cells (ASCs) continuously self-renew in the body column, which is the only competent region for regeneration, while the extremities (tentacles, hypostome, basal disc), made almost exclusively of terminally differentiated cells, fail to rebuild missing structures (David and Plotnick, 1980; Bosch, 2009; David, 2012; Hobmayer *et al.*, 2012; Buzgariu *et al.*, 2014). This suggests that the capacity of a tissue to regenerate requires the presence of ASCs. Three distinct populations of ASCs co-exist in *Hydra*, the myoepithelial stem cells of the epidermis (eESCs), the myoepithelial stem cells of the gastrodermis (gESCs), and the multipotent interstitial stem cells (ISCs) found in the epidermis along the central body column. These three populations, which cannot replace each other, continuously self-renew thus contributing to a dynamic maintenance of tissue homeostasis.

Hydra ASCs behave in a non-classical way, as after mitotic division, they traverse G1 without pausing, then replicate their DNA for about 12 hours to finally pause in G2 (Fig. 2A). However ESCs and ISCs cycle at different paces, ESCs renewing every 3-4 days, ISCs much faster, every 24-30 hours (David and Campbell, 1972; Campbell and David, 1974; Holstein and David, 1990; Buzgariu, *et al.*, 2014), implying a G2 phase duration of about 12 hours for ISCs as compared to several days for ESCs. A recent study suggests that a small fraction of *Hydra* ASCs is maintained quiescent in G2 phase for weeks, contributing to regeneration after bisection (Govindasamy *et al.*, 2014). G2 pausing of stem cells offers several advantages for regeneration (Buzgariu, *et al.*, 2014) (Fig. 2B): (1) it leads to a higher resistance to cell death as observed in *Hydra* as well as mammalian ESCs (Chera, *et al.*, 2009b; Harper *et al.*, 2010), (2) it favors DNA repair (Branzei and Foiani, 2008), (3) upon injury, it allows a rapid entry into mitosis or (4) a possible pre-mitotic differentiation as observed in *Hydra* epithelial cells (Dubel and Schaller, 1990).

In contrast to ESCs that get passively displaced towards the extremities where they terminally differentiate without undergoing mitotic division (Dubel *et al.*, 1987; Dubel and Schaller, 1990), ISCs give rise to migratory progenitors that differentiate post-mitotically,

either locally in the body column or at the extremities (Heimfeld and Bode, 1984; Holstein and David, 1986; Holstein and David, 1990; Hager and David, 1997; Boehm and Bosch, 2012). As a consequence the central region of the animal is highly proliferative while the extremities are built up almost exclusively of terminally differentiated cells. This finely-tuned balance between proliferation and differentiation appears controlled by a series of *c-myc* proto-oncogenes: a limited silencing of *Hymyc1* suffices to reduce ISC proliferation and to increase their differentiation into nematoblasts and gland cells (Hartl *et al.*, 2010; Ambrosone *et al.*, 2012) while *Hymyc2* might play a similar role in ESCs (Hartl *et al.*, 2014). However, the role of cell proliferation in *Hydra* regenerative processes is classically considered as minor (see below).

The pro-regenerative framework

Here we discuss the non-developmental dimensions of regeneration in *Hydra*, i.e the homeostatic conditions that precede injury and constitute *a necessary pro-regenerative framework,* as regeneration is compromised when they are altered. We identified four properties that operate in homeostatic context and provide favorable physiological conditions for a fast and robust induction of regeneration in *Hydra* (Fig. 1): (i) *a high degree of cellular plasticity*, evidenced in both the interstitial and the epithelial cell lineages (Bode *et al.*, 1986; Siebert *et al.*, 2008; Wenger *et al.*, 2016); (ii) *a sustained and localized stemness,* with large stocks of adult stem cells (ASCs) that self-renew in the body column (Bosch *et al.*, 2010); (iii) *a tightly-tuned autophagy flux* in the epithelial cells, which maintains a high level of fitness in response to environmental changes, as evidenced by the loss of regeneration in animals where autophagy is either too high or too low (Chera *et al.*, 2006; Tomczyk *et al.*, 2017), (iv) *a dynamically patterned extracellular matrix* (ECM, named mesoglea in *Hydra*) that supports the translation of the peptidic, metabolic and mechanical signals generated by injuries into a wound response and specific regenerative processes (Sarras, 2012). Together with the developmental dimension of patterning that leads to *de novo* morphogenesis, these properties contribute to define a high regenerative ability.

Cellular plasticity

Plasticity of epithelial stem cells in intact Hydra

To test the respective morphogenetic function of the epithelial and interstitial lineages, reaggregation experiments using epithelial or interstitial cell lineages from strains exhibiting different morphogenetic properties in terms of size, budding rate, were preformed, generating chimeric animals (Marcum and Campbell, 1978a; Sugiyama and Fujisawa, 1978b). The study of such chimeric animals established that epithelial cells largely direct morphogenetic processes. In fact, animals depleted of their stock of ISCs still regenerate. This was shown on animals treated with anti-proliferative agents such as nitrogen-mustard (Diehl and Burnett, 1965), colchicine (Marcum and Campbell, 1978b), hydroxyurea (Sacks and Davis, 1979), and on thermosensitive mutant animals isolated from the field, that lose their stock of ISCs after a pulse heat-shock (Sugiyama and Fujisawa, 1978a; Marcum *et al.*, 1980). In each context, ISCs, interstitial progenitors and subsequently interstitial derivatives are eliminated while the stocks of ESCs are not significantly altered. However, in the so-called "epithelial" animals, the regeneration of apical structures is delayed, the number or size of tentacles often abnormal, and in all cases the nervous system (neurons, nematocytes) missing, so the regenerated head is not functional. Still the morphogenetic processes are active. This important result suggested that the interstitial lineage does not participate in *de novo* head morphogenesis while epithelial cells would play the leading role.

As an alternative, Marcum and Campbell (1978) proposed that the interstitial cells might play an important role in standard regeneration, assuming that their elimination does induce an epithelial adaptation, i.e. a compensatory process where the epithelial cells

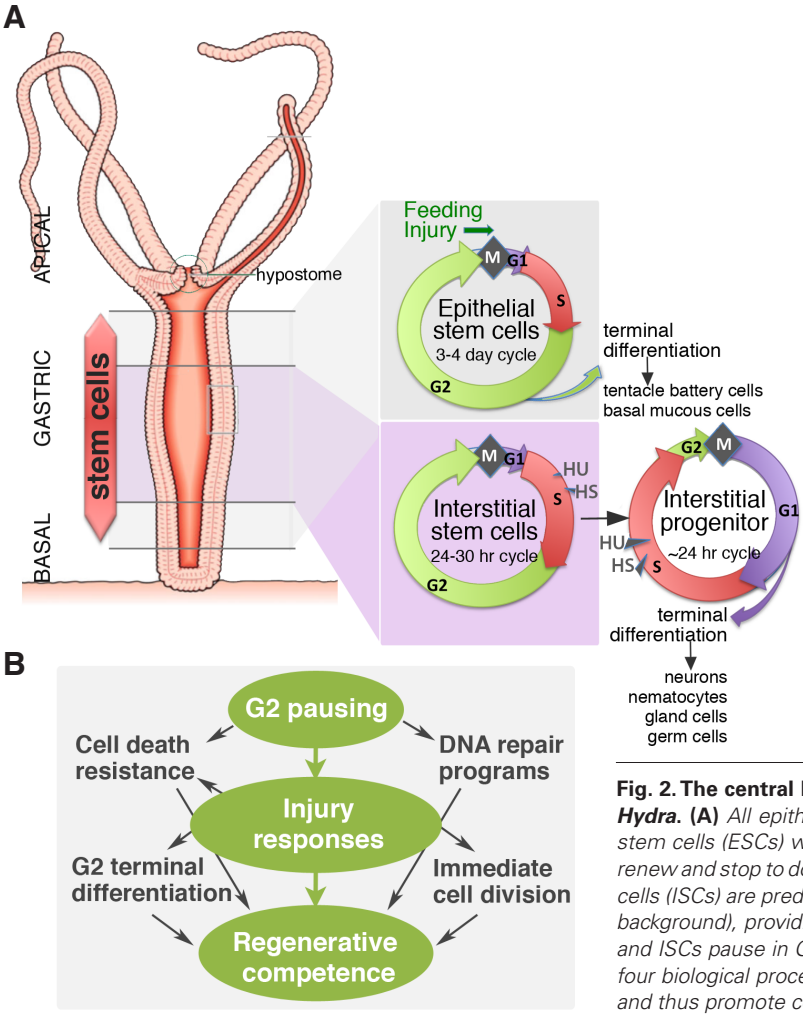

Fig. 2. The central body column can be considered as a pro-blastema in intact *Hydra*. (A) *All epithelial cells, either epidermal or gastrodermal, are considered as stem cells (ESCs) when located along the body column (grey background), i.e. self-renew and stop to do so when displaced towards the extremities. The interstitial stem cells (ISCs) are predominantly found in the central region of the body column (purple background), providing progenitors all along the body column. Note that both ESCs and ISCs pause in G2 and traverse G1 without stopping.* **(B)** *Scheme indicating the four biological processes (written black) which are favored when cells pause in G2, and thus promote competence for regeneration.*

would play some functions usually performed by the interstitial cells. Indeed, epithelial cells can produce morphogenetic substances normally produced by the nerve cells and up-regulate some genes usually expressed in gland and interstitial cells when these cells are eliminated (Schaller *et al.*, 1980; Hornberger and Hassel, 1997). More recently, an analysis based on genome-wide transcriptomics confirmed that epithelial cells adapt to the loss of the interstitial lineage by up-regulating a series of genes normally expressed in cells of the interstitial lineage (Wenger*, et al.*, 2016). Hence, ESCs readily exhibit a property that we name "*epithelial plasticity"*, but the novel functions that ESCs take or modify remain to be characterized.

Dispensable cross-talk between interstitial-derived cells and epithelial cells during head regeneration

The analysis of head-regeneration deficient strains as *reg-16* helped identify the impact of the interstitial cells on the regenerative process. Indeed, the interstitial lineage contributes to regulate *Hydra* regeneration as in *reg-16* animals the repeated depletion of ISCs markedly rescues head regeneration, suggesting a negative impact on the morphogenetic potential of ESCs (Sugiyama and Wanek, 1993). Also the repeated mechanical reopening of the wound stimulates head regeneration in *reg-16* animals (Kobatake and Sugiyama, 1989), suggesting that the local elimination of interstitial cells upon repeated wounding promotes the release of pro-regenerative signals. This situation is indeed reminiscent of the wild-type context, where the injury-induced death of interstitial cells after mid-gastric amputation promotes head regeneration due to the signals released by the dying cells (Chera*, et al.*, 2009b).

By contrast, the gland cells likely play a positive role on head regeneration as observed in the thermosensitive *sf-1* strain, where the loss of ISCs and derivatives after heat-shock progressively alters the efficiency of head regeneration (Guder *et al.*, 2006). Gland cells persist for weeks after the elimination of ISCs and a positive correlation was observed between the persistence of Dickkopf-positive gland cells and head regeneration efficiency. However, in this study the impact of a prolonged starvation was not tested independently of the loss of gland cells. Also gland cells exhibit the ability to transdifferentiate in head-regenerating tissues (Siebert*, et al.*, 2008), and sub-populations of gland cells might play different roles, possibly changing during regeneration. Similarly, nerve cells continuously adopt different phenotypes, as evidenced in animals where ISCs were eliminated (Bode*, et al.*, 1986). For both cell types, the role of transdifferentiation during regeneration is unknown.

In summary, several independent approaches indicate that in wild-type animals maintained in homeostatic conditions, cells from the interstitial lineage prevent the activation of the head regeneration program by inhibiting the morphogenetic potential of the epithelial cells. After bisection, the contribution of interstitial cells on *Hydra* head regeneration appears locally positive, either immediately after mid-gastric bisection through injury-induced cell death, or on the second day when heads form. However, their role is dispensable as epithelial *Hydra* can regenerate heads' anatomies, although not functional heads as mentioned above. Altogether these results suggest that distinct regenerative routes can be taken by the ESCs depending on the presence or the absence of ISCs and interstitial-derived cells. The molecular cross-talk between epithelial cells, ISCs, gland cells and nerve cells during head regeneration is largely unknown, but likely involves Reactive Oxygen Species (ROS) signaling and activation of the innate immune system immediately after injury (Wenger *et al.*, 2014).

The central body column as a homeostatic pro-blastema

Hydra regeneration, which occurs within three days (much faster than in any other animal model), has been classically considered as *morphallactic*, i.e. relying on the reorganization of the pre-existing cells rather than on the production of new cells through cell proliferation. This concept of morphallaxis is supported by several studies that could not identify, for example, a regulation of the number of mitotic figures during head regeneration after decapitation (Park *et al.*, 1970; Hicklin and Wolpert, 1973). However, mitosis is a fast event along the cell cycle (60 to 90 minutes) and recent data obtained after mid-gastric bisection challenged this view (Chera*, et al.*, 2009b; Chera *et al.*, 2011; Buzgariu *et al.*, 2018). Also the monitoring of the S-phase cells detected an enrichment in cycling cells on the second day of regeneration (Holstein *et al.*, 1991; Miljkovic-Licina *et al.*, 2007).

Therefore, we propose to reconsider this morphallactic view by integrating the fact that the central region of the animal might be considered as a pro-blastema where most cycling cells are paused in G2. As all epithelial cells exhibit stem cell properties along the *Hydra* body column, the density of ASCs is high, a situation quite unusual in an adult bilaterian organism. Therefore, the central region of the animal before amputation can be considered as a blastema-like structure we name "pro-blastema", which specifically senses and reacts to the injury signals (Chera*, et al.*, 2009b; Chera*, et al.*, 2011; Buzgariu*, et al.*, 2018). By contrast the apical and basal regions of the body column, which are enriched in cells already committed to differentiate, cannot be considered as a pro-blastema, and might thus react differently to bisection.

During the immediate and early phases of head regeneration (0 – 16 hpa), mitotic division of the interstitial cells appears tightly regulated after mid-gastric bisection (Chera*, et al.*, 2009b; Buzgariu*, et al.*, 2018) but negligible when bisection is performed on "epithelial" animals at higher levels along the axis (Holstein*, et al.*, 1991). During the early-late phase of head regeneration (24 – 36 hpa) a second proliferative event occurs, independent of the bisection level and possibly of the cell lineage. Indeed a wave of cell proliferation is taking place in the presumptive heads on the second day post-bisection, detected in both interstitial and epithelial cells, necessary for head regeneration efficiency and patterning completion (Holstein*, et al.*, 1991; Miljkovic-Licina*, et al.*, 2007). Therefore, two distinct regulations of cell proliferation take place during head regeneration, an early one characterized by injury-induced mitotic events observed after mid-gastric bisection predominantly affecting the interstitial cells, and an early-late one when both interstitial and epithelial cells of the presumptive heads traverse S-phase. The early event is a direct consequence of the pre-injury pro-blastema status of the body column, while the link between the pro-blastema status and the early-late event is currently unclear (Buzgariu*, et al.*, 2018).

Variations in the regenerative response according to the bisection level

Immediately after bisection, the injury response seems to vary with the bisection level, i.e. mid-gastric or sub-tentacular

as injury-induced phosphorylation of the Ribosomal S6 kinase (RSK) is high after mid-gastric bisection but low after decapitation (Kaloulis *et al*., 2004). This is consistent with a strong activation of the MAPK/CREB/RSK pathway in head-regenerating tips after mid-gastric bisection, necessary to launch head regeneration via cell death (Chera*, et al*., 2009b; Chera*, et al*., 2011). Indeed, in wild-type animals bisected at mid-gastric position, a massive wave of cell death affecting almost exclusively the interstitial lineage, occurs immediately after bisection in the head-regenerating tips. These apoptotic cells release signals such as Wnt3 that promote synchronous mitotic events of the surrounding interstitial cells, a process identified as compensatory proliferation induced by cell death (Chera*, et al*., 2009b; Chera*, et al*., 2011; Vriz *et al*., 2014). This wave of mitotic events in the head-regenerating tips can also be traced by flow cytometry (Buzgariu*, et al*., 2018).

In contrast, there is no evidence for a wave of injury-induced cell death and mitotic events in foot-regenerating tips after mid-gastric bisection, or in head-regenerating tips in animals decapitated just below their tentacles (Chera*, et al*., 2009b; Galliot and Chera, 2010), WB, unpublished). These observations suggest that in the immediate-early phase of head regeneration, injury can elicit distinct signaling and cellular responses, mostly morphallactic after decapitation but rather epimorphic after mid-gastric bisection (Buzgariu*, et al*., 2018). A previous study had identified phenotypic differences in head regeneration according to the level of bisection, with tentacle formation preceding hypostome differentiation after decapitation, while the hypostome forms first after basal bisection (Technau and Holstein, 1995). This result points to variations in the regeneration program depending on the bisection level.

The strikingly different properties of the tissues at apical and mid-gastric positions likely explain these two distinct strategies: the upper body column contains epithelial cells that no longer cycle or are going to stop cycling, few ISCs and abundant interstitial progenitors ready to terminally differentiate, while the central gastric region is populated with ASCs, either interstitial or epithelial, as well as cycling interstitial progenitors, and as discussed above, considered as a pro-blastema. Therefore, head regeneration after decapitation appears to take a rather direct route towards differentiation of apical structures from tissues already committed to become apical. By contrast, head regeneration from the mid-gastric level seems to require an early wave of cell division, at least to boost the head regeneration process (Buzgariu*, et al*., 2018). Supporting this idea, an increase in cell proliferation in the early phase of head regeneration appears to accelerate the regeneration process: for example, animals exposed to the nitric acid donor NOC-18 exhibit a sustained increase in cell proliferation already significant from 8 to 16 hours after decapitation, together with an accelerated head regeneration process (Colasanti *et al*., 2009).

Autophagy as a key regulator of animal fitness

Autophagy in slow-aging Hydra vulgaris animals

Several lines of evidence prove that the metabolic status of the animals impact the efficiency of the regenerative process even though regeneration is a highly robust process. Indeed starved animals rapidly lose their ability to bud but maintain their ability to regenerate for weeks, likely because in animals starved for several days the ESCs of both layers enhance their autophagy flux (Chera *et al*., 2009a). Similarly, we suspect that epithelial autophagy plays an important role in the maintenance of regeneration in nerve-less animals that have lost the ability to self-feed, and thus undergo a prolonged period of starvation that induces a highly dynamic autophagy flux that maintains an active metabolism in intact animals.

Two studies showed the importance of maintaining the autophagy flux well balanced to keep *Hydra* regeneration efficient. We first reported the loss of regeneration when autophagy is excessive, as observed in animals where *Kazal1,* a gene encoding a protease inhibitor, is silenced. *Kazal1(RNAi)* animals obtained by repeatedly feeding the animals with dsRNAs, initially maintain head regeneration as in control conditions but after multiple exposures to dsRNAs, likely the time the Kazal1 protein is depleted, no longer survive the amputation stress and die within few hours (Chera*, et al*., 2006). Therefore Chera *et al.,* concluded that *"silencing Kazal1 expression in the body column and the regenerating stumps does not affect the head-formation process per se but rather the conditions that are necessary to survive the amputation stress*". In a loss-of-function approach, we tested the role of autophagy on regenerating *H. vulgaris* by knocking-down *WIPI2*, one key component of the autophagy machinery. The partial silencing of this component suffices to induce an irreversible loss of fitness and to lower the efficiency of regeneration (Tomczyk*, et al*., 2017).

Autophagy in fast-aging sexual Hydra oligactis animals

We also identified a link between autophagy, slow aging and maintenance of regeneration in *H. oligactis (Ho)* animals (Tomczyk*, et al*., 2017). These polyps that remain asexual and fit when maintained at 18°C, start forming gonads within two weeks after transfer to 10°C. In parallel they progressively exhibit a decline of most physiological functions such as feeding, contractility, and regeneration, which becomes completely inefficient after one month at 10°C, while all sexually-mature animals eventually die within four months (Brien, 1953; Yoshida *et al*., 2006; Tomczyk *et al*., 2015; Tomczyk*, et al*., 2017). This aging phenotype shares similarities with the mammalian aging process, namely a loss of all somatic ASCs, either interstitial or epithelial, a progressive sarcopenia and a deficient neurogenesis. To further characterize the origin of the loss of regeneration, another *H. oligactis* strain was used; this strain named "cold resistant" (*Ho_CR*) similarly undergoes sexual differentiation upon temperature drop but then reverts to an asexual stage and survives (Tomczyk*, et al*., 2015). By comparison with animals of the aging strain, named "cold sensitive" (*Ho_CS*), the *Ho_CR* animals maintain their regenerative potential (Fig. 3).

This comparative analysis between the *Ho_CS* and *Ho_CR* strains showed a progressive reduction of ASC self-renewal in aging *Ho_CS* animals, indicating a rapid reduction in somatic ISCs and a slower disappearance of ESCs. By contrast ESCs in sexual *Ho_CR* do maintain their self-renewal. As ESCs are also responsible for maintaining an appropriate autophagy flux, the regulation and intensity of the autophagy flux was compared in *Ho_CS*, *Ho_CR* and *H. vulgaris* animals with a biosensor transiently expressed in intact live animals. This comparative analysis identified a low inducibility of the autophagy flux after starvation or proteasome inhibition in *Ho_CS* when compared to *Ho_CR* or *H. vulgaris.* Given the key role of proteostasis maintenance in mammalian aging (Lopez-Otin *et al*., 2013), these results suggest that the low inducibility of autophagy recorded in *Ho_CS* may take part in the aging process, including the loss of regeneration (Tomczyk*, et al*., 2017).

In summary these studies, performed in slow- and fast-aging

Hydra, show the importance of maintaining the autophagy flux well balanced to keep regeneration efficient, as a highly dynamic autophagy flux appears essential for adapting ESC self-renewal to the environmental conditions. This link between stem cell renewal and autophagy was also identified in hematopoietic stem cells in mammals (Warr *et al.*, 2013), possibly reflecting an ancestral mechanism.

A complex and dynamically patterned extracellular matrix (mesoglea) in *Hydra*

An additional essential pro-regenerative dimension present in intact *Hydra* relies on the biophysical and biochemical properties of the extracellular matrix (ECM) named mesoglea. The mesoglea is a complex extracellular structure that forms an acellular layer all along the animal body between the epidermis and gastrodermis, maintaining the cohesion between the two layers through tight cell-ECM interactions (Sarras, 2012). Such interactions play an essential role in cell migration (Zhang and Sarras, 1994; Stidwill and Christen, 1998), but also in cell differentiation (as the differentiation of battery cells in the tentacles) and regeneration (Fowler *et al.*, 2000). The components of the mesoglea, which exhibits a typical collagen-based structure, are produced by the epithelial cells of both layers (Epp *et al.*, 1986). The up-regulation of some fibrillar collagens (similar to vertebrate type-I, type-II and type-IV) is required for head regeneration as evidenced by gene silencing (Deutzmann *et al.*, 2000; Fowler, *et al.*, 2000). In fact, the mesoglea is patterned along the apical basal axis of the animal with specific components enriched at each pole, such as the meprin-like astacin metalloproteinase HMP2 at the basal pole involved in foot differentiation (Yan *et al.*, 2000a) and the secreted astacin metalloproteinase HMP1 at the apical pole required for *de novo* head formation (Yan *et al.*, 2000b). Hence localized components of the mesoglea play an active role in maintaining the shape of the animal and in the *de novo* patterning of the apical or basal structures that regenerate after amputation (Sarras, 2012).

In the hydrozoan jellyfish *Podocoryne* changes in cellular-ECM interactions appear critical for the stability of the differentiated status and as a consequence for cellular plasticity (Schmid *et al.*, 1999). When ECM is treated with degrading enzymes, striated muscle cells located in the subumbrella plate of the bell can

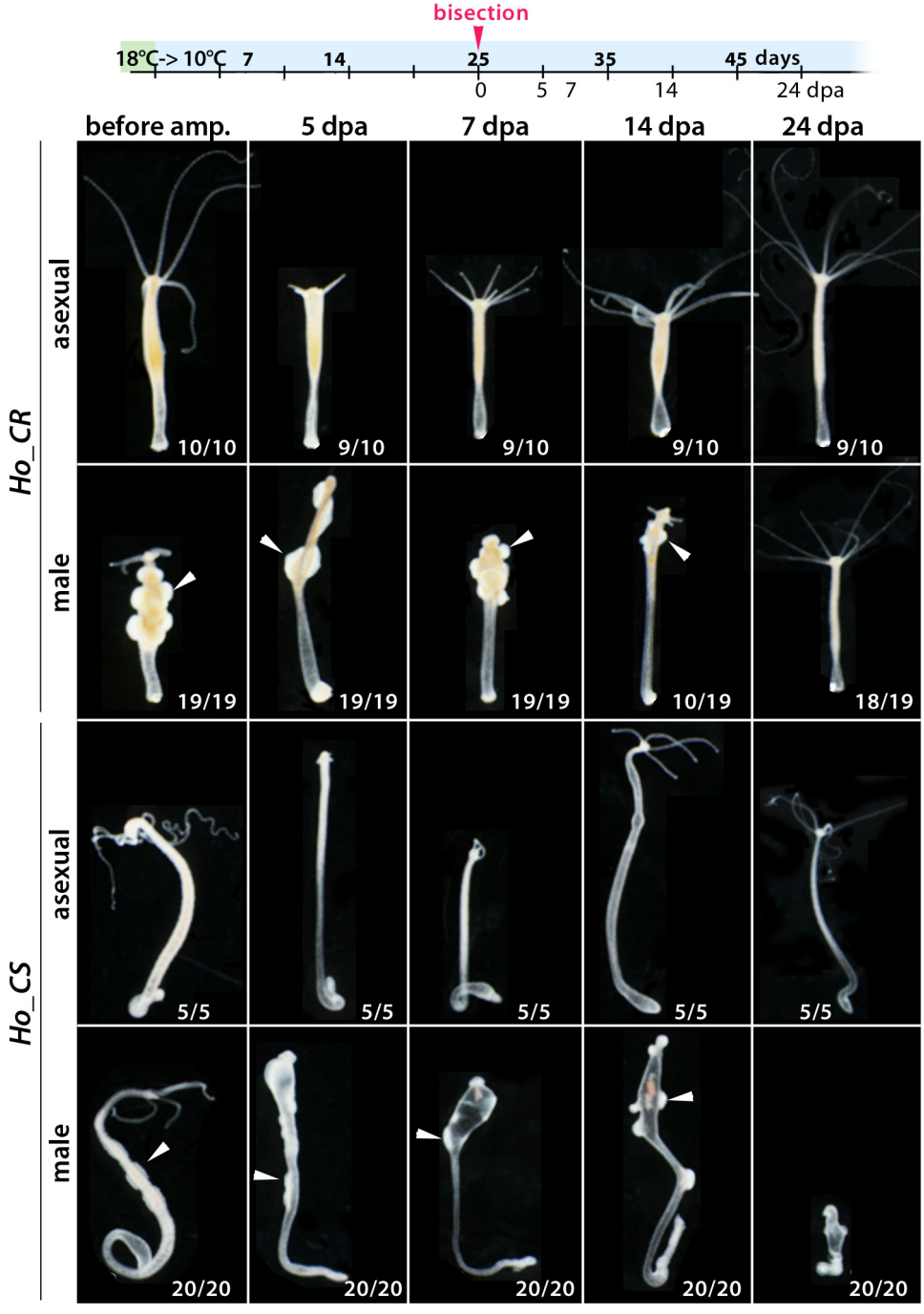

Fig. 3. Loss of head regeneration in aging *Hydra oligactis (Ho).* *Animals of the* Ho_CR *and* Ho_CS *strains readily undergo sexual differentiation, here spermatogenesis, when transferred from 18°C to 10°C, as evidenced by the presence of testes (white arrowheads) that differentiate within three weeks after transfer. At that time, animals were selected as asexual or male and bisected at day-25. Head regeneration was then monitored over 24 days, ratios indicate the number of animals displaying the depicted phenotype. Animals from both strains do regenerate if they remain asexual. Spermatogenesis delays head regeneration by about 10 days in* Ho_CR *and leads to an increased number of abnormal regenerated structures (not shown). By contrast* Ho_CS *animals that show a similar number of testes, undergo aging and are unable to regenerate their head.*

transdifferentiate into at least eight different cell types (smooth muscle cells, nerve cells, nematoblasts, nematocytes, secretory cells, gland cells, digestive cells, interstitial cells) and regenerate *in vitro* some appendage of the jellyfish such as the manubrium (Schmid and Alder, 1984). Disruption of ECM integrity appears to derepress DNA replication in differentiated cells and thus favors the transdifferentiation process (Schmid and Reber-Muller, 1995).

In *Hydra* the regeneration of complete polyps from a mass of re-aggregated cells following polyp dissociation offers a nice paradigm to study the different roles of the mesoglea (Gierer*, et al.*, 1972). The two epithelial cell layers can even be separated by procaine treatment, then dissociated and reaggregated separately, forming epidermal and gastrodermal aggregates that are put in contact to follow the cell behaviors during the reorganization of a bilayered sheet in the presence or the absence of mesoglea (Kishimoto *et al.*, 1996; Murate *et al.*, 1997). Such elegant experiments showed that the mesoglea is neither necessary for the initial adhesion between the two epithelial aggregates, nor for the epibolic movements of the epidermal cells over the gastrodermal aggregate. In fact after tissue dissociation, epithelial cells of the gastrodermis completely lose their polarity, and do not regain it until they get in contact with epidermal epithelial cells; next they rapidly form the typical bilayered sheet, before undergoing patterning processes that lead to axis formation and head differentiation (Kishimoto*, et al.*, 1996; Murate*, et al.*, 1997).

By monitoring the re-establishment of epithelial junctions during reaggregation, Seybold *et al.,* confirmed that the mesoglea is dispensable during the first stages of the reaggregation process, i.e. cell sorting, establishment of the septa and homotypic gap junctions that lead to the apico-basal polarization of both epithelia, a period when the barrier against the external environment is restored (Seybold *et al.*, 2016). However, the differentiation of the basal apparatus, i.e. the formation of desmosomes and hemidesmosomes junctions, and the basal positioning of the actin fibers, requires the presence of the mesoglea. The actomyosin fibers in the epithelial cells exhibit a tightly controlled spatial organization that drives the morphogenetic processes generated by mechanical forces in intact as well as regenerating *Hydra* (Livshits *et al.*, 2017). The connections between the epithelial cytoskeleton and the mesoglea explain its morphogenetic impact.

Conclusions and perspectives

We discuss here four non-developmental dimensions of adult regeneration, which provide the framework for an efficient regeneration. Each dimension can prevent regeneration when altered, but the hierarchy or the interactions between these dimensions are largely unknown and need to be investigated. We do not claim that this list is exhaustive as other dimensions might be at work in *Hydra*. For example, the migratory property of some cell types that accumulate around the wound is not discussed here as no functional link was established with *Hydra* regeneration (Fujisawa *et al.*, 1990; Chera*, et al.*, 2009b; Boehm and Bosch, 2012). Similarly, we did not discuss the homeostatic mechanisms that keep regeneration tightly controlled as a non-oncogenic proliferative process, i.e. the reestablishment of the initial structure without any overgrowth or metastatic process. This pro-regenerative framework identified in *Hydra* might be of interest for regeneration in other organisms and several evolutionary aspects need to be further investigated: Is it possible to characterize the non-developmental dimensions of regeneration in every animal model? How much is shared between the different pro-regenerative frameworks in the different models of regeneration? Is it possible to induce regeneration in organisms with a low regenerative potential just by modulating these non-developmental dimensions? These are some of the questions we open here.

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References

AMBROSONE A, MARCHESANO V, TINO A, HOBMAYER B and TORTIGLIONE C. (2012). Hymyc1 downregulation promotes stem cell proliferation in Hydra vulgaris. *PLoS One* 7: e30660.

BODE H, DUNNE J, HEIMFELD S, HUANG L, JAVOIS L, KOIZUMI O, WESTERFIELD J and YAROSS M. (1986). Transdifferentiation occurs continuously in adult hydra. *Curr Top Dev Biol* 20: 257-280.

BOEHM AM and BOSCH TC. (2012). Migration of multipotent interstitial stem cells in Hydra. *Zoology* 115: 275-282.

BOSCH TC. (2007). Why polyps regenerate and we don't: towards a cellular and molecular framework for Hydra regeneration. *Dev Biol* 303: 421-433.

BOSCH TC. (2009). Hydra and the evolution of stem cells. *Bioessays* 31: 478-486.

BOSCH TC, ANTON-ERXLEBEN F, HEMMRICH G and KHALTURIN K. (2010). The Hydra polyp: nothing but an active stem cell community. *Dev Growth Differ* 52: 15-25.

BRADSHAW B, THOMPSON K and FRANK U. (2015). Distinct mechanisms underlie oral vs aboral regeneration in the cnidarian Hydractinia echinata. *eLife* 4: e05506.

BRANZEI D and FOIANI M. (2008). Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol* 9: 297-308.

BRIEN P. (1953). La Perennite Somatique. *Biol Rev Camb Philos Soc* 28: 308-349.

BROUN M and BODE HR. (2002). Characterization of the head organizer in hydra. *Development* 129: 875-884.

BROWNE EN. (1909). The production of new hydranths in hydra by the insertion of small grafts. *J Exp Zool* 7: 1-37.

BUZGARIU W, CRESCENZI M and GALLIOT B. (2014). Robust G2 pausing of adult stem cells in Hydra. *Differentiation* 87: 83-99.

BUZGARIU W, WENGER Y, TCACIUC N, CATUNDA-LEMOS AP and GALLIOT B. (2018). Impact of cycling cells and cell cycle regulation on Hydra regeneration. *Dev Biol* 433: 240-253.

CAMPBELL RD and DAVID CN. (1974). Cell cycle kinetics and development of Hydra attenuata. II. Interstitial cells. *J Cell Sci* 16: 349-358.

CHANDRAMORE K, ITO Y, TAKAHASHI S, ASASHIMA M and GHASKADBI S. (2010). Cloning of noggin gene from hydra and analysis of its functional conservation using *Xenopus laevis* embryos. *Evol Dev* 12: 267-274.

CHERA S, DE ROSA R, MILJKOVIC-LICINA M, DOBRETZ K, GHILA L, KALOULIS K and GALLIOT B. (2006). Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human SPINK1 pancreatic phenotype. *J Cell Sci* 119: 846-857.

CHERA S, BUZGARIU W, GHILA L and GALLIOT B. (2009a). Autophagy in Hydra: a response to starvation and stress in early animal evolution. *Biochim Biophys Acta* 1793: 1432-1443.

CHERA S, GHILA L, DOBRETZ K, WENGER Y, BAUER C, BUZGARIU W, MARTINOU JC and GALLIOT B. (2009b). Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. *Dev Cell* 17: 279-289.

CHERA S, GHILA L, WENGER Y and GALLIOT B. (2011). Injury-induced activation of the MAPK/CREB pathway triggers apoptosis-induced compensatory proliferation in hydra head regeneration. *Dev Growth Differ* 53: 186-201.

COLASANTI M, MAZZONE V, MANCINELLI L, LEONE S and VENTURINI G. (2009). Involvement of nitric oxide in the head regeneration of Hydra vulgaris. *Nitric Oxide* 21: 164-170.

DAVID CN and CAMPBELL RD. (1972). Cell cycle kinetics and development of Hydra attenuata. I. Epithelial cells. *J Cell Sci* 11: 557-568.

DAVID CN and PLOTNICK I. (1980). Distribution of interstitial stem cells in Hydra. *Dev Biol* 76: 175-184.

DAVID CN. (2012). Interstitial stem cells in Hydra: multipotency and decision-making. *Int J Dev Biol* 56: 489-497.

DEUTZMANN R, FOWLER S, ZHANG X, BOONE K, DEXTER S, BOOT-HANDFORD RP, RACHEL R and SARRAS MP, JR. (2000). Molecular, biochemical and functional analysis of a novel and developmentally important fibrillar collagen (Hcol-I) in hydra. *Development* 127: 4669-4680.

DIEHL FA and BURNETT AL. (1965). The Role of Interstitial Cells in the Maintenance of Hydra. 3. Regeneration of Hypostome and Tentacles. *J Exp Zool* 158: 299-317.

DUBEL S, HOFFMEISTER SA and SCHALLER H. (1987). Differentiation pathways of ectodermal epithelial cells in hydra. *Differentiation* 35: 181-189.

DUBEL S and SCHALLER HC. (1990). Terminal differentiation of ectodermal epithelial stem cells of Hydra can occur in G2 without requiring mitosis or S phase. *J Cell Biol* 110: 939-945.

EPP L, SMID J and TARDENT P. (1986). Synthesis of the mesoglea by ectoderm and endoderm in reassembled Hydra. *J Morphol* 189: 271-279.

FOWLER SJ, JOSE S, ZHANG X, DEUTZMANN R, SARRAS MP, JR. and BOOT-HANDFORD RP. (2000). Characterization of hydra type IV collagen. Type IV collagen is essential for head regeneration and its expression is up-regulated upon exposure to glucose. *J Biol Chem* 275: 39589-39599.

FUJISAWA T, DAVID CN and BOSCH TC. (1990). Transplantation stimulates interstitial cell migration in hydra. *Dev Biol* 138: 509-512.

GALLIOT B and CHERA S. (2010). The Hydra model: disclosing an apoptosis-driven generator of Wnt-based regeneration. *Trends Cell Biol* 20: 514-523.

GALLIOT B. (2012). Hydra, a fruitful model system for 270 years. *Int J Dev Biol* 56: 411-423.

GIERER A, BERKING S, BODE H, DAVID CN, FLICK K, HANSMANN G, SCHALLER H and TRENKNER E. (1972). Regeneration of hydra from reaggregated cells. *Nat New Biol* 239: 98-101.

GOVINDASAMY N, MURTHY S and GHANEKAR Y. (2014). Slow-cycling stem cells in hydra contribute to head regeneration. *Biol Open* 3: 1236-1244.

GUDER C, PINHO S, NACAK TG, SCHMIDT HA, HOBMAYER B, NIEHRS C and HOLSTEIN TW. (2006). An ancient Wnt-Dickkopf antagonism in Hydra. *Development* 133: 901-911.

HAGER G and DAVID CN. (1997). Pattern of differentiated nerve cells in hydra is determined by precursor migration. *Development* 124: 569-576.

HARPER LJ, COSTEA DE, GAMMON L, FAZIL B, BIDDLE A and MACKENZIE IC. (2010). Normal and malignant epithelial cells with stem-like properties have an extended G2 cell cycle phase that is associated with apoptotic resistance. *BMC Cancer* 10: 166.

HARTL M, MITTERSTILLER AM, VALOVKA T, BREUKER K, HOBMAYER B and BISTER K. (2010). Stem cell-specific activation of an ancestral myc protooncogene with conserved basic functions in the early metazoan Hydra. *Proc Natl Acad Sci USA* 107: 4051-4056.

HARTL M, GLASAUER S, VALOVKA T, BREUKER K, HOBMAYER B and BISTER K. (2014). Hydra myc2, a unique pre-bilaterian member of the myc gene family, is activated in cell proliferation and gametogenesis. *Biol Open* 3: 397-407.

HEIMFELD S and BODE HR. (1984). Interstitial cell migration in Hydra attenuata. I. Quantitative description of cell movements. *Dev Biol* 105: 1-9.

HICKLIN J and WOLPERT L. (1973). Positional information and pattern regulation in hydra: the effect of gamma-radiation. *J Embryol Exp Morphol* 30: 741-752.

HOBMAYER B, RENTZSCH F, KUHN K, HAPPEL CM, VON LAUE CC, SNYDER P, ROTHBACHER U and HOLSTEIN TW. (2000). WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* 407: 186-189.

HOBMAYER B, JENEWEIN M, EDER D, EDER MK, GLASAUER S, GUFLER S, HARTL M and SALVENMOSER W. (2012). Stemness in Hydra - a current perspective. *Int J Dev Biol* 56: 509-517.

HOLSTEIN T and DAVID CN. (1986). The properties of nerve cell precursors in hydra. *Dev Biol* 115: 18-26.

HOLSTEIN TW and DAVID CN. (1990). Cell cycle length, cell size, and proliferation rate in hydra stem cells. *Dev Biol* 142: 392-400.

HOLSTEIN TW, HOBMAYER E and DAVID CN. (1991). Pattern of epithelial cell cycling in hydra. *Dev Biol* 148: 602-611.

HOLSTEIN TW, HOBMAYER E and TECHNAU U. (2003). Cnidarians: an evolutionarily conserved model system for regeneration? *Dev Dyn* 226: 257-267.

HORNBERGER MR and HASSEL M. (1997). Expression of HvRACK1, a member of the RACK1 subfamily of regulatory WD40 proteins in Hydra vulgaris, is coordinated between epithelial and interstitial cells in a position-dependent manner. *Dev Genes Evol* 206: 435-446.

JOUBIN K and STERN CD. (2001). Formation and maintenance of the organizer among the vertebrates. *Int J Dev Biol* 45: 165-175.

KALOULIS K, CHERA S, HASSEL M, GAUCHAT D and GALLIOT B. (2004). Reactivation of developmental programs: The cAMP-response element-binding protein pathway is involved in hydra head regeneration. *Proc Natl Acad Sci USA* 101: 2363-2368.

KISHIMOTO Y, MURATE M and SUGIYAMA T. (1996). Hydra regeneration from recombined ectodermal and endodermal tissue. I. Epibolic ectodermal spreading is driven by cell intercalation. *J Cell Sci* 109 (Pt 4): 763-772.

KOBATAKE E and SUGIYAMA T. (1989). Genetic analysis of developmental mechanisms in hydra. XIX. Stimulation of regeneration by injury in the regeneration-deficient mutant strain, reg-16. *Development* 105: 521-528.

LENGFELD T, WATANABE H, SIMAKOV O, LINDGENS D, GEE L, LAW L, SCHMIDT HA, OZBEK S, BODE H and HOLSTEIN TW. (2009). Multiple Wnts are involved in Hydra organizer formation and regeneration. *Dev Biol* 330: 186-199.

LIVSHITS A, SHANI-ZERBIB L, MAROUDAS-SACKS Y, BRAUN E and KEREN K. (2017). Structural Inheritance of the Actin Cytoskeletal Organization Determines the Body Axis in Regenerating Hydra. *Cell Rep* 18: 1410-1421.

LOPEZ-OTIN C, BLASCO MA, PARTRIDGE L, SERRANO M and KROEMER G. (2013). The hallmarks of aging. *Cell* 153: 1194-1217.

MARCUM BA and CAMPBELL RD. (1978a). Developmental roles of epithelial and interstitial cell lineages in hydra: analysis of chimeras. *J Cell Sci* 32: 233-247.

MARCUM BA and CAMPBELL RD. (1978b). Development of Hydra lacking nerve and interstitial cells. *J Cell Sci* 29: 17-33.

MARCUM BA, FUJISAWA T and SUGIYAMA T. (1980). A mutant hydra strain (sf-1) containing temperature-sensitive interstitial cells, in *Developmental and Cellular Biology of Coelenterates.* (eds. P Tardent and R Tardent) Elsevier/North Holland, Place, 429-434.

MILJKOVIC-LICINA M, CHERA S, GHILA L and GALLIOT B. (2007). Head regeneration in wild-type hydra requires de novo neurogenesis. *Development* 134: 1191-1201.

MURATE M, KISHIMOTO Y, SUGIYAMA T, FUJISAWA T, TAKAHASHI-IWANAGA H and IWANAGA T. (1997). Hydra regeneration from recombined ectodermal and endodermal tissue. II. Differential stability in the ectodermal and endodermal epithelial organization. *J Cell Sci* 110 (Pt 16): 1919-1934.

NAKAMURA Y, TSIAIRIS CD, OZBEK S and HOLSTEIN TW. (2011). Autoregulatory and repressive inputs localize Hydra Wnt3 to the head organizer. *Proc Natl Acad Sci USA* 108: 9137-9142.

PARK HD, ORTMEYER AB and BLANKENBAKER DP. (1970). Cell division during regeneration in Hydra. *Nature* 227: 617-619.

PFEFFERLI C and JAZWINSKA A. (2015). The art of fin regeneration in zebrafish. *Regeneration (Oxf)* 2: 72-83.

REINHARDT B, BROUN M, BLITZ IL and BODE HR. (2004). HyBMP5-8b, a BMP5-8 orthologue, acts during axial patterning and tentacle formation in hydra. *Dev Biol* 267: 43-59.

RENTZSCH F, GUDER C, VOCKE D, HOBMAYER B and HOLSTEIN TW. (2007). An ancient chordin-like gene in organizer formation of Hydra. *Proc Natl Acad Sci USA* 104: 3249-3254.

SACKS PG and DAVIS LE. (1979). Production of nerveless Hydra attenuata by hydroxyurea treatments. *J Cell Sci* 37: 189-203.

SARRAS MP, JR. (2012). Components, structure, biogenesis and function of the Hydra extracellular matrix in regeneration, pattern formation and cell differentiation. *Int J Dev Biol* 56: 567-576.

SCHALLER HC, RAU T and BODE H. (1980). Epithelial cells in nerve-free hydra produce morphogenetic substances. *Nature* 283: 589-591.

SCHMID V and ALDER H. (1984). Isolated, mononucleated, striated muscle can undergo pluripotent transdifferentiation and form a complex regenerate. *Cell* 38: 801-809.

SCHMID V and REBER-MULLER S. (1995). Transdifferentiation of isolated striated muscle of jellyfish in vitro: the initiation process. *Semin Cell Biol* 6: 109-116.

SCHMID V, ONO SI and REBER-MULLER S. (1999). Cell-substrate interactions in cnidaria. *Microsc Res Tech* 44: 254-268.

SEYBOLD A, SALVENMOSER W and HOBMAYER B. (2016). Sequential development of apical-basal and planar polarities in aggregating epitheliomuscular cells of Hydra. *Dev Biol* 412: 148-159.

SIEBERT S, ANTON-ERXLEBEN F and BOSCH TC. (2008). Cell type complexity in the basal metazoan Hydra is maintained by both stem cell based mechanisms and transdifferentiation. *Dev Biol* 313: 13-24.

STIDWILL RP and CHRISTEN M. (1998). Alteration of fibronectin affinity during differentiation modulates the *in vitro* migration velocities of Hydra nematocytes. *Cell Motil Cytoskeleton* 41: 68-73.

SUGIYAMA T and FUJISAWA T. (1978a). Genetic analysis of developmental mechanisms in Hydra. II. Isolation and characterization of an interstitial cell-deficient strain. *J Cell Sci* 29: 35-52.

SUGIYAMA T and FUJISAWA T. (1978b). Genetic analysis of developmental mechanisms in hydra. V. Cell lineage and development of chimera hydra. *J Cell Sci* 32: 215-232.

SUGIYAMA T and WANEK N. (1993). Genetic analysis of developmental mechanisms in hydra. XXI. Enhancement of regeneration in a regeneration-deficient mutant strain by the elimination of the interstitial cell lineage. *Dev Biol* 160: 64-72.

TANAKA EM and REDDIEN PW. (2011). The cellular basis for animal regeneration. *Dev Cell* 21: 172-185.

TARDENT P. (1963). Regeneration in the Hydrozoa. *Biol Rev* 38: 293-333.

TECHNAU U and HOLSTEIN TW. (1995). Head formation in Hydra is different at apical and basal levels. *Development* 121: 1273-1282.

TOMCZYK S, FISCHER K, AUSTAD S and GALLIOT B. (2015). Hydra, a powerful model for aging studies. *Invertebr Reprod Dev* 59: 11-16.

TOMCZYK S, SCHENKELAARS Q, SUKNOVIC N, WENGER Y, EKUNDAYO K, BUZGARIU W, BAUER C, FISCHER K, AUSTAD S and GALLIOT B. (2017). Deficient autophagy drives aging in Hydra. *BioRxiv.* DOI: 10.1101/236638

TREMBLEY A. (1744). *Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes.* (Verbeck, Leiden; 1744).

VOGG MC, WENGER Y and GALLIOT B. (2016). How somatic adult tissues develop organizer activity. *Curr Top Dev Biol* 116: 391-414.

VOGG MC, BECCARI L, IGLESIAS OLLÉ L, PERRUCHOUD C, RAMPON C, VRIZ S, WENGER Y and GALLIOT B. (2018). An evolutionarily-conserved Wnt3/β-catenin / Sp5 feedback loop module restricts head organizer in Hydra. *BioRxiv*: DOI: 10.1101/265785.

VRIZ S, REITER S and GALLIOT B. (2014). Cell death: a program to regenerate. *Curr Top Dev Biol* 108: 121-151.

WARR MR, BINNEWIES M, FLACH J, REYNAUD D, GARG T, MALHOTRA R, DEBNATH J and PASSEGUE E. (2013). FOXO3A directs a protective autophagy program in haematopoietic stem cells. *Nature* 494: 323-327.

WEBSTER G and WOLPERT L. (1966). Studies on pattern regulation in hydra. I. Regional differences in time required for hypostome determination. *J Embryol Exp Morphol* 16: 91-104.

WENGER Y, BUZGARIU W, REITER S and GALLIOT B. (2014). Injury-induced immune responses in Hydra. *Semin Immunol* 26: 277-294.

WENGER Y, BUZGARIU W and GALLIOT B. (2016). Loss of neurogenesis in Hydra leads to compensatory regulation of neurogenic and neurotransmission genes in epithelial cells. *Philos Trans R Soc B* 371: 20150040.

YAN L, FEI K, ZHANG J, DEXTER S and SARRAS MP, JR. (2000a). Identification and characterization of hydra metalloproteinase 2 (HMP2): a meprin-like astacin metalloproteinase that functions in foot morphogenesis. *Development* 127: 129-141.

YAN L, LEONTOVICH A, FEI K and SARRAS MP, JR. (2000b). Hydra metalloproteinase 1: a secreted astacin metalloproteinase whose apical axis expression is differentially regulated during head regeneration. *Dev Biol* 219: 115-128.

YAO T. (1945). Studies on the organizer problem in Pelmatohydra oligactis. I. The induction potency of the implants and the nature of the induced hydranth. *J Exp Biol* 21: 145-150.

YOSHIDA K, FUJISAWA T, HWANG JS, IKEO K and GOJOBORI T. (2006). Degeneration after sexual differentiation in hydra and its relevance to the evolution of aging. *Gene* 385: 64-70.

ZHANG X and SARRAS MP, JR. (1994). Cell-extracellular matrix interactions under *in vivo* conditions during interstitial cell migration in Hydra vulgaris. *Development* 120: 425-432.

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