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# Cellular and Molecular Mechanisms of *Hydra* Regeneration

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## Abstract

Regeneration of lost body parts is essential to regain the fitness of the organism for successful living. In the animal kingdom organisms from different clades exhibit varied regeneration abilities. *Hydra* is one of the few organisms that possess tremendous regeneration potential, capable of regenerating complete organism from small tissue fragments or even from dissociated cells. This peculiar property has made this genus one of the most invaluable model organisms for understanding the process of regeneration. Multiple studies in *Hydra* led to the current understanding of gross morphological changes, basic cellular dynamics and the role of molecular signalling such as the Wnt signalling pathway. However, cell-to-cell communication by cell adhesion, role of extracellular components such as extracellular matrix (ECM), nature of cell types that contribute to the regeneration process need to be explored in-depth. Additionally, roles of developmental signalling pathways need to be elucidated to enable more comprehensive understanding of regeneration in *Hydra.* Further research on cross communication among extracellular, cellular and molecular signalling in *Hydra* will advance the field of regeneration biology. Here, we present a review of the existing literature on *Hydra* regeneration biology and outline the future perspectives.

### Keywords

Hydra; Regeneration; Morphallaxis; Extracellular matrix; Signalling and Pattern formation

## 1 Introduction

Replacement of lost body parts or tissues is an extraordinary phenomenon. The demonstration of the ability to regenerate missing parts after amputation in an animal by Trembley is the earliest record of this phenomenon. He called this animal *Hydra*, after a Greek mythological serpent which can regenerate multiple heads upon decapitation. This initiated a great interest in search for animals with regeneration abilities by biologists. Regeneration of damaged or diseased tissue or body part is important for successful survival of the organism. Therefore, it has fascinated the field of biomedical science for its potential applications and has led to the emergence of regenerative medicine.

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Occurrence of regeneration process varies in different animal phyla (Table 1). In certain animals, ability of regenerating the whole organism is observed and in many others it is restricted to specific organs or tissue types. Whole body regeneration ability is evident from the earliest divergent multicellular organisms such as sponges (Ayling 1983; Borisenko et al. 2015). Members of the phylum Cnidaria, which are early divergent animals with defined body column and cell types similar to bilaterians, also exhibit regeneration ability (Schmid and Tardent 1971; Bossert et al. 2013). Especially the fresh water polyp *Hydra* exhibits tremendous regeneration capacity and it is the earliest record of animal regeneration (Trembley 1744). In Bilateria, members of basal phylum Platyhelminthes which belong to free living flatworm groups possess the whole organismal regeneration ability (Egger et al. 2007; Ritter and Congdon 1900; Stevens and Boring 1905; Monti 1900; Graff 1882; Dalyell 1814; Ruhl 1927; Spallanzani 1769). Until now there is no evidence of whole body regeneration in Ecdysozoa (Arthropoda and Nematoda) (Bely and Nyberg 2010). However, regeneration of limb has been well studied in Arthropoda and this ability varies across the taxa with good regeneration abilities observed in crustaceans (Bohn 1970; Minelli et al. 2013). In annelids, regeneration of anterior and posterior parts has been shown with varying capacity while the regeneration potential is completely absent in some animals like leeches (Bely 2006; Hyman 1940). In Mollusca, siphonophores of bivalves (Meyer and Byers 2005) and arms of cephalopods (Tressler et al. 2014) can regenerate and this ability is not conserved to the same extent in all the members of the phylum. Echinoderms are interesting invertebrates which can regenerate most complex organs/body-parts and this ability is well conserved in the phylum (Cuénot 1948; Goss 1969; Hyman 1955; Carnevali 2006).

Chordates show different degrees of regeneration potential across the phylum. Among these, urochordates, especially ascidians such as *Botrylloides violaceus* (complete body) (Berrill 1951; Brown et al. 2009) and *Ciona intestinalis* (partial body) (Dahlberg et al. 2009; Auger et al. 2010) display considerable regeneration capacities. Within the Pisces, selected members were reported to exhibit regeneration (Unguez 2013; Goss 1969; Broussonet 1786) and recent studies in zebrafish have demonstrated the ability to regenerate different organs and fins (Johnson and Weston 1995). In Amphibia, salamanders such as newt and axolotl can regenerate lost limb (a relatively a complex structure) and this property is very unique and not reported in any other vertebrates (Spallanzani 1769; Goss 1969). Noticeable regeneration power has been demonstrated in lizard tail (Reptilia) and they are the closest animals to mammals that exhibit significant replacement potential of complex tissue (structure) (Lozito and Tuan 2017; Simpson 1964; Bellairs and Bryant 1985; Bryant and Bellairs 1967; Etheridge 1967). Aves (Stone and Rubel 2000; Cotanche 1987) and Mammals (Iismaa et al. 2018) possess very poor regeneration abilities and can regenerate relatively simple tissue types in their adult life. They cannot regenerate more complex structures such as appendages. An interesting exception is found in many species of deer which can regenerate their antlers (Goss 1983).

The regeneration ability was lost in different animals which are closely related or belonging to sister clades of organisms which exhibit this property. Many hypotheses such as adaptive, epiphenomenal, proximate causes etc. have been proposed to explain the loss of regeneration ability. However, most of these cannot be applied universally and thus provide no satisfactory explanation (Goss 1963; Wagner and Misof 1992; Goss 1992).

Among the mentioned animals with varied regeneration capacities only a few representative members have gained popularity. Invertebrate model systems such as *Hydra* and planaria are widely used for understanding the whole organism regeneration. Whereas zebrafish and axolotl are used as vertebrate model systems to understand the regeneration potential limited to few structures or organs. Apart from the regeneration abilities (*Hydra* and planaria) and phylogenetic closeness to mammals (zebrafish and axolotl) these also have other major advantages as mentioned below.

1. Established protocols and ease of maintaining in controlled laboratory conditions.
2. Amenable to genetic manipulations using transgenic technology and siRNA mediated gene expression knockdown.
3. Availability of genome, transcriptome and other molecular information.
4. Concerted efforts and sharing of reagents by active research groups.

In this chapter, we aim to review the current understanding of the regeneration process in *Hydra*, the oldest regeneration model system. We believe it is important to consolidate the recent findings and compare them in light of present understanding in other regenerative model systems which will also help us identify future directions for the studies to unravel the complex process of regeneration. Especially, *in vivo* model systems are ideal due to limited success of research focused majorly on stem cell based regenerative therapies. Further, since tissue regeneration is a complex phenomenon that depends on different cell types and extracellular factors/niche, it is important to investigate regeneration at an organism level. Previous reports have provided significant insights into the organismal regeneration. These studies suggest that the formation of signalling centre is a critical step in the regeneration process. Such signalling centres called ‘organizers’ were reported in axis induction of *Hydra* and newt embryo. The organizer comprises of a group of cells that emit signals and regulate the patterning of neighbouring tissue. However, the cells that contribute to formation of signalling centre and the process of evoking tissue pattering required for regeneration remain elusive. Due to these reasons, organisms such as *Hydra* and planaria which can exhibit complete organismal regeneration serve as important model systems.

## 2 Hydra

*Hydra* is a fresh water living organism and belongs to the class Hydrozoa of phylum Cnidaria (Marques and Collins 2004; Campbell 1987). Cnidaria is a sister group to bilateria and an early divergent phylum comprising multicellular animals with tissue level organization (Fig. 1A). Members of the phylum are radially symmetrical and evolved with two germ layers. Animals from this phylum share bilaterian cell types such as neurons and muscular epithelial cells (Fig. 1B). These properties have positioned the members of these phylum as favourite models to understand the molecular evolution determining organized body plan and evolution of cell types conserved in bilateria.

*Hydra* is a diploblast possessing two layers of the cells which form a cylindrical body containing a gastric lumen. These polyps are among the earliest animals to have evolved a

defined body plan and has an oral-aboral axis with the oral end consists of a dome shaped hypostome (mouth) being surrounded by a ring of 5-6 tentacles which help them in feeding. The body column of a polyp can be divided into a gastric region, a budding region and a peduncle region. The aboral end of the polyp is called basal disk which can secrete mucus that helps in the attachment of *Hydra* to various substrates (Hoffmeister and Schaller 1985). As seen in Figure 1B, the cell layers are mainly constituted of epithelial cells with either ectodermal or endodermal stem cell origin. The epithelial cells have myofibrils at the base, due to which they are called as epitheliomuscular cells (Hess et al. 1957). These myofibrils are oriented perpendicular to each other in ectoderm and endoderm. This organization of myofibrils helps in contraction or elongation of the polyp (Passano and McCullough 1964). Endodermal cells are ciliated and have phagocytic capabilities while the ectodermal cells secrete a glycocalyx layer on the outermost side of the cells to protect the polyp from environment (Hess et al. 1957). Apart from epithelial cells, *Hydra* also possess multipotent interstitial stem cells located in the interstitial spaces of epithelial cells. These stem cells differentiate into cells with special functions such as gland cells, mucus cells, neuronal cells, nematocytes and gametes (oocytes or sperms) (Campbell and David 1974). The ectoderm and endoderm are divided by an acellular mesoglea which is essentially the extracellular matrix (ECM) (Sarras Jr et al. 1993).

## 3 Gross morphological changes during *Hydra vulgaris* regeneration

*Hydra* can regenerate missing body parts upon transverse or longitudinal amputation (Fig. 2a). The *Hydra* polyps are also capable of regenerating from re-aggregated cells. These polyps when dissociated into single cells can reorganize and regenerate into a whole polyp when these cells are pelleted (Fig. 2b). The cells reorganize into a lumen within first 12 h with ectodermal cells outside and endodermal cells facing inside the lumen (Technau and Holstein 1992; Gierer et al. 1972). This reorganization (possibly) arises from differential cell adhesion between ectodermal and endodermal cells (Technau and Holstein 1992). This process is followed by establishment of head organizer *de novo* within 18-30 h (Sato et al. 1990). First appearance of the head and tentacle structures can be seen by 48-72 h and finally the whole adult *Hydra* body forms within 4 to 7 days (Technau and Holstein 1992).

In case of regeneration in response to amputation, the site of injury responds by re-organization of the epithelial cells to close the wound within an hour. This is then followed by lack of any gross morphological changes until 30 h post amputation. After 30-36 h, small tentacle buds begin to emerge from the regenerating tip beginning the process of morphological differentiation of cells. The emergence of the tentacles happens over the next 24 h with the completion of the whole process taking place by 72 h, where the tentacles are mature enough to catch the prey. On the other hand, basal disk regeneration is completed within 30-36 h (Hoffmeister and Schaller 1985). During regeneration wound healing is the initial step towards the morphological changes leading to major the cellular reorganization. This requires remodelling of ECM to facilitate tissue morphogenesis.

## 4 ECM dynamics during axis patterning and regeneration

ECM in animals has varied functions like cell-adhesion, mechanical support, cell-signalling, scaffolding for other extracellular molecules etc. The transition of unicellular organism to multicellular organisms requires ECM as a conduit for integration of various information between cells to function together. Recent studies have shown that the basic 'Toolkit' required to form the basal membrane coincided with emergence of multicellularity. ECM components have been found to be encoded in eukaryotes as early as the unicellular choanoflagellate *Monosiga brevicollis.* More recently, it was reported that a unicellular organism named *Ministeria vibrans* belonging to a filozoan clade of filasterea which diverged earlier to Choanozoa contains a gene similar to collagen IV (Grau-Bove et al. 2017). This premetazoan origin of collagen type IV indicates that it is possibly the most primitive form of collagens and possibly other components of ECM evolved from this. Earliest phylum reported to have a complete functional set of ECM components and related proteins is Cnidaria. The fact that ECM components expanded their repertoire from phylum Cnidaria onwards indicates their eumetazoan specific functions. Hence, studying cnidarian ECM can shed light on the role of ECM components in the organization of the body plans of early multicellular organisms.

ECM has been reported to play a crucial role in regulating cell fate and tissue morphology in multiple organisms. The chemical and physical properties of ECM can influence cell behaviour and fate. It was reported that solely by varying the stiffness properties ECM, the differentiation fate of mesenchymal stem cells could be altered (Engler et al. 2006). The regulation of cell fate by ECM occurs as a function of its interaction with ECM specific cell receptors such as integrins which in turn transmit the information either through chemical signal transduction or mechanically through force distribution via actomyosin networks (Mao and Baum 2015). ECMs can also regulate tissue morphogenesis by controlling and sequestering morphogenic ligands including BMP (Nistala et al. 2010). These capabilities of ECM can therefore make them an important player in 'sculpting' an organism during embryogenesis. A recent study showed that during *Drosophila* embryogenesis, ECM components in the basement membrane are differentially deposited to form a morphogen-like gradient during tissue elongation (Crest et al. 2017). This creates an anisotropic resistance to isotropic tissue expansion and hence helps in shaping organs by creating a mechanical imbalance. Another study in sea urchins showed how spatio-temporal incorporation of the hygroscopic chondroitin sulphate proteoglycan causes swelling of ECM which creates hydrostatic pressure for folding of epithelial sheet during embryonic invagination (Lane et al. 1993). The branching morphogenesis in mice salivary gland has been shown to be regulated by fibronectin deposition which signals the tightly packed epithelial cells to lose the e-cadherin contacts and help in conversion of cell-cell adhesions to cell— matrix adhesions (Sakai et al. 2003).

In mammalian systems, ECM has been reported to be crucial for regeneration and sustaining cell proliferation and differentiation post injury. Mammals possess very limited potency to regenerate their lost or injured tissues. Skeletal muscles are among the few tissues that mammals can regenerate and it was found that upon muscle degeneration, it leaves behind a hull of basement membrane ECM which acts as a scaffold to facilitate myofiber fusion

(Vracko and Benditt 1972). In mammals, early stages of skeletal muscle regeneration are associated with downregulation of laminin and collagen type I to facilitate de-differentiation of satellite cells to form myoblasts (Gulati et al. 1983). Fibronectin, Hyaluronic acid and Tenascin-C are shown to be up-regulated during the same period. Another study showed that by altering the ECM stiffness of a 3 day old mouse, it was possible to restore the ability to regenerate heart which is lost after day 1 (Notari et al. 2018). Certain species of mammals are reported to have an astonishing capability to regenerate. For example, the African spiny mouse (*Acomys* sp.) which can loose and regenerate up to 60% of its dorsal surface including hair, follicle, skin, sweat glands etc. Here, It was shown that laminin and collagen type I molecules are down-regulated while the cells de-differentiate and later upregulated during cellular differentiation (Seifert et al. 2012). Another study which looked at the regeneration of skeletal muscle of tibialis anterior in *Acomys* found that it has higher collagen type VI in the regenerating tissue as compared to *Mus* (Maden et al. 2018). This difference renders a softer biomechanical property to the *Acomys* tissue which is believed to be important to achieve a faster, scar-free regeneration with higher macrophage infiltration, low expression of inflammatory and fibrotic genes at the site of injury. A recent study has demonstrated that fibronectin in the niche was necessary for maintaining a functional muscular stem cell population capable of regenerating lost muscular tissue. The reconstitution of fibronectin levels in aged stem cell niche is enough to remobilize and restore functional muscular stem cells (Lukjanenko et al. 2016).

In axolotls, skeletal muscle regeneration follows similar regulation of collagen type I, laminins, fibronectin, hyaluronic acid and tenascin-C as in mammals in the early phase of regeneration to allow de-differentiation of satellite cells (Mailman and Dresden 1976; Tassava et al. 1996; Contreras et al. 2009). In *Xenopus* tadpoles, it has been reported that Hyaluronic acid plays an important role in regeneration of tail bud after amputation (Contreras et al. 2009). Adequate level of hyaluronic acid in the regenerating tail bud is required for sustaining mesenchymal cell proliferation.

Studies in zebrafish also indicate a similar role of ECM in regeneration. Fin regeneration studies indicate an early upregulation of fibronectin, hyaluronic acid and tenascin-C which seems to be a conserved mechanism across different organisms in the vertebrates (Contreras et al. 2009). Additionally, Hapln1a, hyaluronan and proteoglycan aggrecan are also upregulated during the process. On the other hand, laminin levels do not show any appreciable changes during fin regeneration in zebrafish. Fibronectin has been shown to be necessary for the heart regeneration and is shown to be expressed by epicardial cells. The cardiomyocytes starts to express itgb3 receptors which can respond to presence of fibronectin in the site of injury for a successful heart regeneration (Wang et al. 2013). A study on the caudal fin regeneration indicate a crucial role of collagen type IV and specific matrix remodellers from the MMP and TIMP family of proteins (Bai et al. 2005).

*Hydra* mesoglea is 0.5 - 2 μm thick depending upon its position in the body. The mesoglea is a trilaminar structure dividing the two epithelia (Davis and Haynes 1968). The epithelial cells are in direct contact with the basal lamina which sandwiches a thick central layer of interstitial matrix. The mesoglea also has trans-mesogleal pores which enable inter-epithelial cellular interactions via cell processes running through them (Shimizu et al. 2008). ECM

secretory dynamics have been studied using the *Hydra* regeneration model. It is known that post amputation there is a retraction of Mesoglea, and its restoration is required for initiation of regeneration. Post-amputation, loss of ECM is followed by sealing of wound which causes direct contact of ectoderm and endoderm cells, leading to change of morphology of these cells from being cuboidal or columnar to flattened (Fig. 3) (Shimizu et al. 2008; Shimizu et al. 2002). During regeneration, within 3 hours, all the components of ECM are actively transcribed by their respective cells and then by 7 hours components of the basal lamina - endoderm secretes laminin and HyCol IV such that basal lamina is formed and hence leading to regaining the normal cuboidal morphology of the cells. Contact of the basal lamina with receptors of the ectodermal cells results in secretion of components of the interstitial matrix and hence leading to complete formation of mesoglea within 20 hrs post amputation (Sarras Jr 2012). During the regeneration of re-aggregated cells, it is reported that mesoglea formation takes place immediately after reorganization of ectoderm and endoderm cells into a lumen (Technau and Holstein 1992). The mesogleal components are first secreted by 12-17 hrs of aggregation and the maturation of the mesogleal structure is reported to be completed by 48-72 hrs (Sarras Jr et al. 1993). The morphogenetic processes required for regeneration of reaggregated cells succeeds the mesoglea formation and the formation of a mature mesoglea is an absolute necessity for success of regeneration (Sarras Jr et al. 1993). It has also been reported that disruption of any of this process either by knockdown of the ECM components or by inhibiting their biosynthesis chemically or by preventing ECM-receptor interaction, the process of regeneration is arrested (Sarras Jr et al. 1993). Therefore, it seems very plausible that the important role of ECM in regulating regeneration and other tissue function evolved very early during the evolution. As can be noted above, the precise mechanistic role of ECM during regeneration in more complex organisms is unclear despite strong evidence showing its importance. A highly regenerative model system such as *Hydra* with its less complicated tissue system and signalling networks could be crucial to shedding light to ECM mediated regulation. Studies focussing on biomechanical aspects of ECM in *Hydra* could unravel various aspects of their role in physiological regulation and how it evolved to perform more complex function in a system such as human.

## 5 Cellular basis of regeneration

Regeneration in animals can be either for homeostatic cell replacement like the replacement of blood cells or for reparative replacement wherein a damaged tissue or organ needs to be regenerated. In the context of this chapter, discussion will be limited to the latter type. Regeneration can be broadly divided into two types: -

**a)** Epimorphosis is a type of regeneration involving active cell proliferation for the completion of regeneration. This process can further be divided into two types i.e., blastema-mediated or compensatory regeneration. In blastema-mediated regeneration, repair of lost tissue or organ proceeds by recruitment of either pre-existing progenitor cells in the vicinity of the injury or by de-differentiation of differentiated cells from the vicinity. These cells then start proliferating to form a mass of heterogeneous undifferentiated cells at the regenerating tip called blastema. Once a necessary number of cells are amassed, the cells in blastema

start to differentiate and initiate the process of morphogenesis similar to that seen during embryogenesis. This process then culminates with restoration of the lost tissue either completely or partially depending upon the organism e.g. planaria, axolotls etc.(Alvarado 2006). In the compensatory regeneration, the regeneration proceeds without any blastema formation or requirement of stem cells. In this process, differentiated cells from the vicinity are recruited to site of injury and proliferate to replace the lost tissue. Liver regeneration is a prime example of compensatory regeneration (Michalopoulos and DeFrances 1997).

**b)** Morphallaxis is a type of regeneration hallmarked by absence of cellular proliferation. The existing tissue is re-patterned to replace the lost tissue. This kind of regeneration is frequently observed in lower invertebrate organisms such as *Hydra* (Lenhoff et al. 1986; Trembley 1744).

Epimorphic type of regeneration is the most prevalent type of regeneration across the animal kingdom. It would be wrong to assume dividing cells as the sole contributors towards the new tissue during regeneration. There exist possibilities of contribution from trans-differentiated and de-differentiated cells which might contribute to a new population of dividing and differentiating cells (Jopling et al. 2011). Hence to understand how regeneration process is regulated, it becomes important to dissect the origins of cells involved as a source of new cells required for regeneration upon specific injury signals. In planaria, multiple studies have shown that a continuously dividing population of cells called neoblasts contribute to formation of new tissue (Newmark and Sanchez Alvarado 2000). These cells are clonal and pluripotent in nature; have the capability to rescue regeneration deficit planarians to regenerate with as few as 3-5 cells (Wagner et al. 2011). These clonogenic neoblasts are recruited to site of injury where they proliferate into a mass of undifferentiated cells to form blastema. The blastema cells then undergoes differentiation process for patterning the lost tissue (Newmark and Sanchez Alvarado 2000). In vertebrate systems, blastema formation doesn't consist in entirely of one type of clonogenic pluripotent cells like neoblasts. There seems to have a pre-determined heterogeneous group of cells having unipotent or multipotent capabilities. For example, in *Xenopus* the muscle regeneration is solely dependent on dormant population of satellite cells found adjacent to muscle fibres (Le Grand and Rudnicki 2007). These are the muscle stem cells which upon activation, differentiate into muscle fibres. In axolotl, it was shown using lineage tracing experiments that muscular satellite cells and Schwann cells are found to be unipotent and were responsible for muscular and Schwann cells respectively; while, dermis cells of mesodermal origin contribute to a diverse repertoire of cartilage and connective tissues (Kragl et al. 2009). In the zebrafish caudal fin regeneration model, it was shown that most of the cell types required for regenerating the fin were derived from specific unipotent progenitors and the same is the case during the fin development during embryogenesis (Tu and Johnson 2011).

In *Hydra*, regeneration is manifested in two different modes depending on the region of amputation. Any amputation away from mid-gastric region will exhibit morphallactic mode of regeneration. In this mode, the wounded tissue undergoes wound healing by bringing together the ectodermal and endodermal cells which is initiated by the endodermal cells

from severed edge in a fashion similar to blastopore closure (Takaku et al. 2005). This is then followed by cellular re-arrangements and differentiation required for regeneration without any proliferation. It has also been reported that there is no involvement of interstitial stem cells or their derivatives towards the regeneration process and hence only requiring epithelial stem cells for the whole process (Sugiyama and Fujisawa 1978). On the other hand, it was reported that head regeneration after a mid-gastric cut proceeds through a process similar to the epimorphosis (Chera et al. 2009). Upon amputation, there are detectable levels of activation of apoptotic pathway in interstitial cells such as neurons, nematocytes etc. present near the area of injury. These cells start secreting Wnt3a in a yet unknown fashion. These molecules of Wnt ligand then activate the Wnt signalling in nearby interstitial stem cells and induce them to do *compensatory cell proliferation.* This proliferation however does not form a very large mass of cells like in blastema. Simultaneously, the epithelial stem cells which are located at the apical end starts secreting Wnt3a and assume the role of head organizer after which follows the process of regeneration as seen in morphallactic regeneration (Galliot and Chera 2010).

Several studies have shown in *Hydra* that certain strains of *Hydra* do not exhibit any signs of aging or senescence while other strains can be induced to senesce upon induction of gametes (Martínez and Bridge 2012; Kaliszewicz 2018). The immortality in *Hydra* is attributed to the ability of *Hydra* to maintain a steady state of shedding of cells and cell proliferation. The stem cells in the body column divide continuously either to replenish themselves or differentiate into specialized cells. The epithelial cells divide roughly every 3-4 days and interstitial cells divide every 1.5 days (Martínez and Bridge 2012). These cells produce differentiated cells which are pushed to either end of the body column and are sloughed off. The differentiated cells proceed to move towards their destination depending on the origin of the cells. They move to the basal disk if the cells originate from the peduncle region, the tentacles if the originate from region just below the tentacle ring or are pushed to form new bud if the cells originate from region above the budding region and below the tentacle ring. The cells moving towards their respective destinations have variable rate of movement depending on their position. Cells at the base of the tentacle takes on an average of 4 days to reach the tip of the tentacles and being pushed off. The cells at the base of the hypostome and the at the peduncle reach their respective destination on 20 days and the cells in the body column takes about 2-8 days to reach the budding zone and to bud depending on their position with respect to the bud (Fig. 4) (Campbell 1967). In conclusion, it is evident that specific cell types play important role in regeneration and tissue homeostasis.

## 6 Molecular Signalling

For the development of a zygote into an animal, there are seven major signalling pathways that act in a concerted manner and organize the proliferating cells into the final morphology. These different signalling pathways have been classified based on either ligands or signal transducers involved. These are, Wnt/Wingless (Wg), transforming growth factor-β (TGF-β)/BMPs, Notch, RTK, Hedgehog (Hh), JAK-STAT [signal transducers and activators of transcription] pathway) and Nucleic acid receptor/Hormone receptor mediated signalling (Barolo and Posakony 2002; Gerhart 1999). All the signalling pathways work towards a unified goal which is to create axes and break symmetry in the developing embryo. From

early metazoans like sponges that have a single oral-aboral axis to mammals that have multiple axes like dorso-ventral (D-V), anterior-posterior (A-P), left-right (L-R) planes, these pathways are critical in the patterning of all organisms. During the process of development, these signalling pathways act in a spatio-temporally regulated manner to regulate various cellular functions like cell division, migration, differentiation, organization into tissues, recycling cellular components, enabling cells to respond to other signalling pathways and apoptosis (Sanz-Ezquerro et al. 2017). These developmental signalling pathways are also reactivated when an organism must undergo the process of regeneration. The reformation of the body axes and proper patterning of lost tissues is a result of the cumulative action of these pathways.

### 6.1 Injury response

When there is loss of tissues, the first event to occur is the activation of injury mediated signalling response. In multiple organisms such as planaria (Petersen and Reddien 2009), axolotl (Bryant et al. 2017), newt and salamander (Yokoyama 2008), it has been reported that the earliest responses upon wounding were essential for initiation of the cascade of developmental signalling needed to regenerate lost structures. In *Hydra*, following a mid-gastric amputation, the initial ROS signalling responses upregulated by $H_2O_2$ were shown to activate multiple pathways. Majority of these are immune response genes and it has also been shown that multiple signalling pathways such as STK, Pi3K, ERK 1–2, and MAPK pathways that are a part of injury induced responses are necessary for the formation of the head organizer (Arvizu et al. 2006; Tischer et al. 2013) (Fig. 6).

### 6.2 Wnt signalling

The Wnt signalling pathway is highly conserved in evolution and is reported to play roles in proliferation, cell polarity, cell fate determination, migration and apoptosis during the development of both invertebrates and vertebrates (Miller 2002; Loh et al. 2016). This pathway is essentially a short-range communication system that uses the Wnt lipid-modified glycoproteins and has roles in both axis patterning (canonical) and cell-polarity (non-canonical) via two distinct signalling cascades (Alexandre et al. 2014). The canonical pathway is activated when the Frizzled receptors are bound by the Wnt ligands and the coreceptor Lrp5/6 is ligated to it. This leads to inhibition of the GSK3-β kinase which prevents phosphorylation of β-catenin resulting in its stabilization and nuclear localization. Nuclear β-catenin binds to the Tcf/Lef transcription factors and activates the Wnt target genes resulting in determination of cell fate. The non-canonical component of Wnt signalling is often β-catenin independent and via multiple intracellular effector proteins, plays a role in planar cell polarity (PCP), convergent extension (CE) and directed cell migration (Loh et al. 2016).

Wnt signalling is thought to predate the “Hox patterning system” and is possibly therefore the original symmetry breaking mechanism and primary axis organizer (Larroux et al. 2007; Martindale 2005). Traditionally, Wnt has been considered as a posterior organizer signal although there exists a debate due to the expression of few Wnt targets that play a role in sensory epithelium differentiation in the oral region of pre-bilaterian organisms (Nichols et al. 2006; Ryan et al. 2013).

The different components of the Wnt signalling pathway are differentially present in various animals across the animal phyla. Even in basal metazoans like sponges, it has been observed that the multiple Wnt ligands express in a polarized manner across the larvae from the anterior to posterior extremes (Windsor Reid et al. 2018; Adamska et al. 2007; Adamska et al. 2010). In the ctenophore *Mnemiopsis leidyi*, it was observed that the Wnt ligands were expressed in the aboral pole, tentacles and the apical organ indicating their role in polarized pattern formation (Pang et al. 2010). In *Nematostella*, the presence of multiple Wnt ligands and their conservation at sequence level has been shown (Rigo-Watermeier et al. 2012). In *Nematostella*, the Wnt/β-catenin signalling is important for patterning the oral-aboral axis during the development of the organism using a cross-talk with the Hox patterning system (Leclere et al. 2016; DuBuc et al. 2018).

In bilaterians, deuterostomes (echinoderms to vertebrates), Wnt signalling has been shown to play a role during early embryogenesis in anterior-posterior patterning (Schubert and Holland 2013; Darras et al. 2018). Similar roles have been demonstrated in protostome clades such as Arthropoda (*Drosophila, Tribolium, Gryllus* and *Achaearanea*)(Nusslein-Volhard and Wieschaus 1980; Bolognesi et al. 2008; Miyawaki et al. 2004; McGregor et al. 2008). Wnt is also known to regulate the *caudal* gene and in turn the Hox patterning system in mouse and zebrafish (Chawengsaksophak et al. 2004; Shimizu et al. 2005). The Hox patterning system is another set of genes conserved from cnidarians important in axis patterning (Ryan et al. 2006; Shenk et al. 1993; Gauchat et al. 2000; Chourrout et al. 2006; Naito et al. 1993; Schummer et al. 1992; Reddy et al. 2015).

Besides playing a role in embryonic development, the Wnt/β-catenin signalling also plays a posteriorizing role in the process of homeostasis and regeneration. In sponges, Wnt signalling was found to be important for regeneration (Windsor Reid et al. 2018). In planaria, it has been observed that the Wnt/β-catenin signalling elicited by wounding is necessary for posterior tissue patterning (Petersen and Reddien 2008, 2009). While the canonical Wnt signalling is necessary for proper posterior regeneration, even anterior regeneration has been shown to require nuclear translocation of β-catenin and signalling through unknown mechanisms (Sureda-Gomez et al. 2016). Among the vertebrates, different Wnt ligands have been shown to act in the regulation of the regeneration process. In zebrafish tailfin regeneration, Wnt/β-catenin signalling acts upstream of FGF-signalling and is necessary for the cell proliferation involved in blastema formation (Kawakami et al. 2006). During heart regeneration in adult zebrafish and juvenile mice, Wnt signalling was observed to be induced upon cardiomyocyte injury and necessary to switch between fibrosis and relatively scar-free regeneration (Ozhan and Weidinger 2015). Wnt signalling is active and multiple Wnt ligands express throughout adulthood in urodele tails. Such an expression pattern suggests multiple roles in urodele tail regeneration as well (Caubit et al. 1997). In axolotl, which can regenerate whole appendages, Wnt signalling has been shown to be necessary (Kawakami et al. 2006). Multiple targets and effectors of the Wnt signalling pathway were found to be differentially regulated and were implicated in the lizard tail regeneration (Hutchins et al. 2014).

In *Hydra*, the Wnt signalling pathway was identified to be a key player in the organization of head, bud formation and head regeneration in *Hydra* (Hobmayer et al. 2000; Bode 2003;

Broun et al. 2005). A total of 11 Wnt ligands have been identified and six of them have been shown to be a part of the organizer centre of the *Hydra* hypostome (Lengfeld et al. 2009) (Fig. 5). There were also autoregulatory mechanisms discovered to localise the Wnt expression to the hypostome (Nakamura et al. 2011). Systemic activation of the Wnt signalling pathway results in ectopic tentacle formation all over the body column of *Hydra*, turning the whole organism into a head like structure confirming its role in head patterning (Broun et al. 2005). The regulation of Hox genes by Wnt signalling was observed in *Hydra* and these were also upregulated during the process of head regeneration (Reddy et al. 2015; Schummer et al. 1992) (Fig. 5). Multiple components of Wnt signalling have been shown exhibit dynamic expression pattern during *Hydra* head regeneration (Petersen et al. 2015) (Fig. 6).

### 6.3 TGF-beta superfamily signalling

The TGF-β superfamily is a very large group of molecules that play a vital role in development of metazoans. The canonical form of signal transduction is through Ser-Thr kinases to intracellular mediator class of Smad proteins. The superfamily of ligands consists of more than 30 members including TGF-βs, BMPs, GDFs, Activin and Nodal (Feng and Derynck 2005; Kitisin et al. 2007). The effector Smad proteins are classified into 3 classes, the R-Smads, Co-Smads and the Anti-Smads based on their structure and function. The R-Smads including Smad-1, 2, 3, 5 and 8 are the receptor regulated Smads and are directly phosphorylated by binding of the Activins, BMPs and TGF-β ligands. The co-Smads or the common Smads include one member, the Smad 4 and are required for all distinct pathways as they form heteromeric complexes with R-Smads and translocate to the nucleus for target gene activation.

The anti-Smads include the Smad 6 and 7 classes and inhibit signalling by the R-Smads and co-Smads by stably associating with the receptors (Kawabata and Miyazono 1999).

The components of the TGF-β signalling pathway are conserved across metazoans starting from placozoans (Huminiecki et al. 2009). The members of the TGF-β superfamily are morphogens generating gradients and playing an important role in both development of body axes and tissues patterning. Nodal and BMPs initiate regulatory loops in the zygote to form the first embryonic axis. While the Wnt signalling pathway plays an important role in the formation of the antero-posterior axis, the TGF-β members play a critical role in the development of the dorso-ventral (D-V) axis of the organism (Wu and Hill 2009). Components of the TGF-β pathway have been identified in the ctenophore, *Mnemiopsis leidyi*, and play a role in morphogenesis but not in early axis patterning (Pang et al. 2011). TGF-β members specify the dorsal regions of a developing embryo in both invertebrate (arthropods) and vertebrate (*Xenopus* and zebrafish) systems (De Robertis and Kuroda 2004; O'Connor et al. 2006).

In different classes of echinoderms, there are studies reporting TGF-β members playing a role in regeneration of the arms (Bannister et al. 2005; Patruno et al. 2003; Patruno et al. 2002). In the zebrafish heart regeneration, TGF-β signalling pathway plays a role in switching between the scar-based repair to cardiomyocyte-based regeneration (Chablais and Jaźwińska 2012; Choi et al. 2013; Dogra et al. 2017). The TGF-β/ALk4/Smad3 pathway has

been shown to play important role in the spinal cord development during regeneration (Casari et al. 2014). TGF-β has been shown to play a role in cell proliferation, activation of the BMP and ERK signalling cascades and reversible prevention of wound epithelium resulting in the formation of regeneration structures (Ho and Whitman 2008; Beck et al. 2003). This signalling pathway is also critical for initiation and control of limb regeneration in axolotl (Levesque et al. 2007).

Multiple studies have elucidated the role of BMP signalling in body patterning and regeneration in *Hydra.* A BMP5-8 homolog was identified in *Hydra* and was shown to play a role both in tentacle formation and foot patterning during regeneration (Reinhardt et al. 2004) (Fig. 5). A chordin-like protein has been reported to play a conserved role in the formation of the head organiser during budding and regeneration (Rentzsch et al. 2007) (Fig. 5). The Nodal signalling pathway via *Pitx* in *Hydra* has been shown to play a role in breaking the radial symmetry during the process of budding and allow for the organizer pathways to form a bud on one side of the body column (Watanabe et al. 2014) (Fig. 5). This suggests a possible role in organizer formation during head regeneration. This is further supported by a report of multiple TGF ligands being downregulated during regeneration at transcript level and the upregulation of Smads (Petersen et al. 2015) (Fig. 6).

### 6.4 Notch signalling

The Notch signalling pathway is a mediator of cell-cell communication in a juxtracrine manner and plays a crucial role in embryonic development. Like other signalling pathways, it controls cell proliferation, differentiation and cell fate determination during embryonic development in spatio-temporal manner (Liao et al. 2016). A very important function of the Notch signalling pathway is the ability to form boundaries within developing tissues. The Notch signalling pathway is one of the simplest with very few components in its core network. The canonical Notch signalling pathway consists of a Notch transmembrane receptor that contains a Notch intracellular domain (NICD) and is activated by transmembrane ligands like Delta and Serrate/Jagged on neighbouring cells. This leads to the cleavage of the NICD which moves to the nucleus and regulates transcription of target genes (Kopan 2012). The lack of an amplification mechanism in this signalling pathway makes the stoichiometry and the signalling strength, which can be modulated by post-translational modifications, important for manifestation of the effect of the signalling pathway. Such dosage-dependent action and consequences have been studied in *Drosophila*, mice and human diseases (Andersson et al. 2011). Across the animal kingdom, this pathway has been shown to play a role in the specification of germ layers and differentiation of cells into specific subtypes (Shi and Stanley 2006). Notch signalling has a well characterized role in the development of nervous system and this is conserved from sponges (Richards et al. 2008; Richards and Degnan 2012). In *Nematostella*, disruption of the Notch signalling pathway affects the development of neurons and cnidocytes and indicates a conserved ancient origin for the multifunctional sensory cells/neurons (Marlow et al. 2012). In amphioxus embryos, Notch signalling was shown to be co-opted into the anterior-posterior boundary patterning of the developing somites (Onai et al. 2015). The role of the Notch signalling pathway during early mouse embryonic development in axial mesoderm

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formation, ectodermal anterior-posterior patterning and neural tube formation has also been established (Souilhol et al. 2015).

Notch signalling components (like *delta-1)* were among the genes that have been shown to be upregulated in a translation independent manner, early in the regeneration of planarians (Wenemoser et al. 2012). This is necessary in maintaining the midline identity of the planaria (Sasidharan et al. 2017). Notch signalling is necessary for zebrafish tail-fin regeneration in the formation of a blastema (Münch et al. 2013; Grotek et al. 2013). In addition to the tail-fin, Notch signalling has been shown to be critical for the proliferation of cardiomyocytes and atrial to ventricular trans-differentiation during heart regeneration (Zhao et al. 2014; Zhang et al. 2013; Raya et al. 2003). In the *Xenopus* tadpole it was shown that the Notch signalling pathway, acting downstream of BMP signalling, is required and positively correlates with regenerative capacity (Beck et al. 2003).

The Notch signalling pathway is also conserved in *Hydra* and was shown to govern differentiation of cells originating from the interstitial stem cell lineage of the polyps (Kasbauer et al. 2007). It also plays an important role in determining the boundaries in *Hydra* polyps. These boundaries include the ones between tentacles and the head region and between the newly formed bud and the parent polyp (Munder et al. 2013). The process of boundary formation between tissues/cells is an important part of development and determines the morphogenetic processes. The Notch target is expressed just before the foot cells begin to differentiate at the boundary between the bud and the parent polyp. This signalling was necessary for the completion of the bud development and detachment from the parent polyp (Munder et al. 2010). The Notch signalling pathway in *Hydra* is necessary for the formation of the *Wnt3a* expressing head organizer during the regeneration of *Hydra.* Further, inhibition of Notch signalling led to the loss of boundary between head and tentacle and repression of the head formation. This finally ended in non-regenerating tips because of perturbed spatio-temporal expression of head and tentacle specific genes and networks (Munder et al. 2013).

### 6.5 RTK signalling

The receptor tyrosine kinases (RTKs) are autocatalytic receptors that function by phosphorylating various tyrosine residues. All RTKs consist of an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic tyrosine kinase domain which harbours serine, threonine and tyrosine residues that can be phosphorylated. When the ligand binds, the receptors dimerize or undergo allosteric transitions leading to activation. The phosphorylation of the receptors recruits other cytoplasmic adaptor proteins via multiple interaction domains. The signals are then transmitted via a network of cytoplasmic proteins to the nucleus resulting in activation of various target genes (Volinsky and Kholodenko 2013).

In planarians, the FGF signalling and Wnt signalling circuits are juxtaposed and regulate the anterior-posterior axis patterning (Scimone et al. 2016). Among other growth factors, the FGFs have been shown to play a critical role in regeneration in few organisms. EGFR-3 was shown to be important for the asymmetric cell divisions that take place in planarian neoblasts upon near-lethal irradiation and was important for regeneration of these animals by

regulating the size of the blastema produced (Fraguas et al. 2011; Lei et al. 2016). This signalling was also necessary for proper gut regeneration and homeostasis in planarians (Barberán et al. 2016). During the process of homeostasis of fins in adult zebrafish

, the blastema markers including *shh, msxb* and *mkp3* are expressed at low levels indicating that regenerative processes are important for the maintenance of the fins in these animals. Removal of Fgfs resulted in drastic atrophy of all fin types and showed a critical role for these growth factors in preservation of dermal bone, joint structures and all supporting tissues (Wills et al. 2008). Fgfs are also necessary for the vascularisation of the regenerating heart in zebrafish (Scimone et al. 2016). The FGFs were also shown to play a role in lizard tail regeneration (Fraguas et al. 2011; Lei et al. 2016).

Sponges have 9 members belonging to the RTK family (Suga et al. 2001). In *Hydra*, 15 RTKs belonging to eight families have been identified, including 3 ephrin family members (Reddy et al. 2011). In *Hydra*, an FGFR called *kringelchen* has been shown to express at the boundary between parent polyp and bud. Additionally, multiple components of the FGFR signalling pathway have been shown to be essential for bud detachment by modulating the actin cytoskeleton at that location (Sudhop et al. 2004). Homologs of VEGF and FGF have been identified in *Hydra* and VEGF has been shown to have roles in bud formation and regeneration indicating a conserved role in tube formation and branching morphogenesis (Krishnapati and Ghaskadbi 2013). *HyEph1* has been shown to be upregulated during the later stages of regeneration during which its role in boundary formation could be necessary (Tischer et al. 2013). The members of RTK signalling pathway in *Hydra* and their expression during regeneration have been characterized, however, their functional significance has not been established yet.

## 7 Conclusion and Future perspectives

Studies on the early morphological changes during *Hydra* head regeneration have revealed changes in cell shape and cell-cell contacts established between ecto- and endo-dermal cells. However, the significance of these cell-cell contacts remains elusive. Therefore, a focused approach is required to analyse the molecular mechanism underlying this cell-cell contact during early stages of regeneration.

Another important component that appears to play crucial role in regeneration is the ECM. It retracts immediately after amputation facilitating the contact of outer ectoderm and inner endoderm. Additionally, experiments perturbing the ECM components indicate its role in the regeneration process. The questions that still remain unanswered are, what are the changes occurring in ECM in response to damage and how these changes are read or communicated to neighbouring cells? Such studies will also be important in understanding the molecular mechanism of wound healing and tissue repair in higher organisms.

Formation of a signalling centre (organizer) is important for successful regeneration. The nature of cell types that are competent and form the organizer is not understood. This needs to be addressed to gain insight into the cellular contribution of regeneration process.

The role of signalling pathways such as the Wnt and the TGF-β superfamily are relatively well characterized. At the same time molecular regulation of other developmental signalling pathways such as hedgehog, Hippo etc. are not studied. Understanding the role of these signalling mechanisms and their cross talk will prove invaluable towards elucidation of the regeneration process.

The signalling pathways lead to transcriptional changes in the cell required for both developmental and regenerative processes. To bring about these changes, there are multiple epigenetic regulators that are recruited by or recruit transcription factors to target regions. Very few studies have investigated the role of histone modifiers, modifications and chromatin remodellers in the process of regeneration. The conservation of histones in *Hydra* (Reddy et al. 2017) and *Hydractinia* (Török et al. 2016) has been established. Since the histones are conserved their modification machinery, at least in part may also be conserved. Therefore, *Hydra* can be used as a model organism to study the epigenetic mechanism of regeneration and identify differences with other organisms that cannot regenerate. This will facilitate in identifying the presence or absence of epigenetic blockade in animals with no regeneration ability.

In *Hydra*, head regeneration process is well studied whereas regeneration of foot is not addressed extensively. Comparing the head and foot regeneration process might provide clues towards a common molecular mechanism necessary for the regeneration process.

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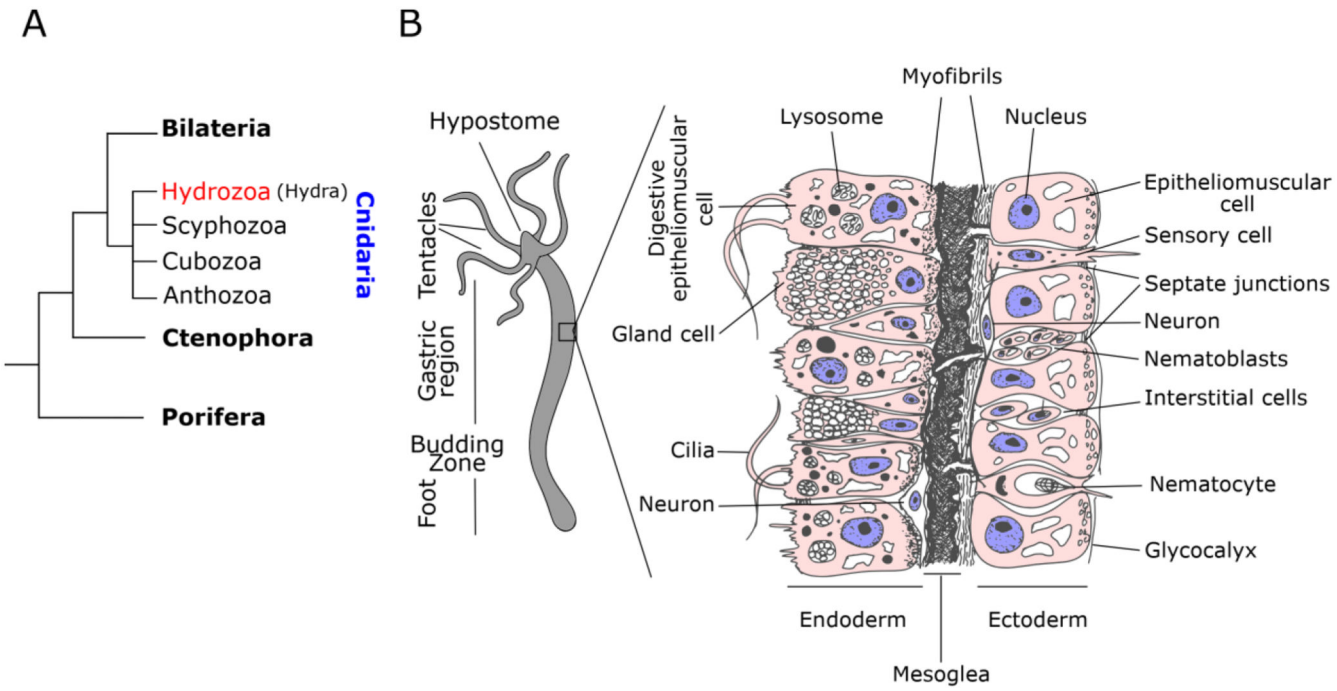


**Figure 1. Phylogenetic position of *Hydra* and general morphology**

A)Phylogenetic relation of basal metazoans and different classes of phylum Cnidaria. B) General body plan and histology cartoon depiction with germ layers and cell types.




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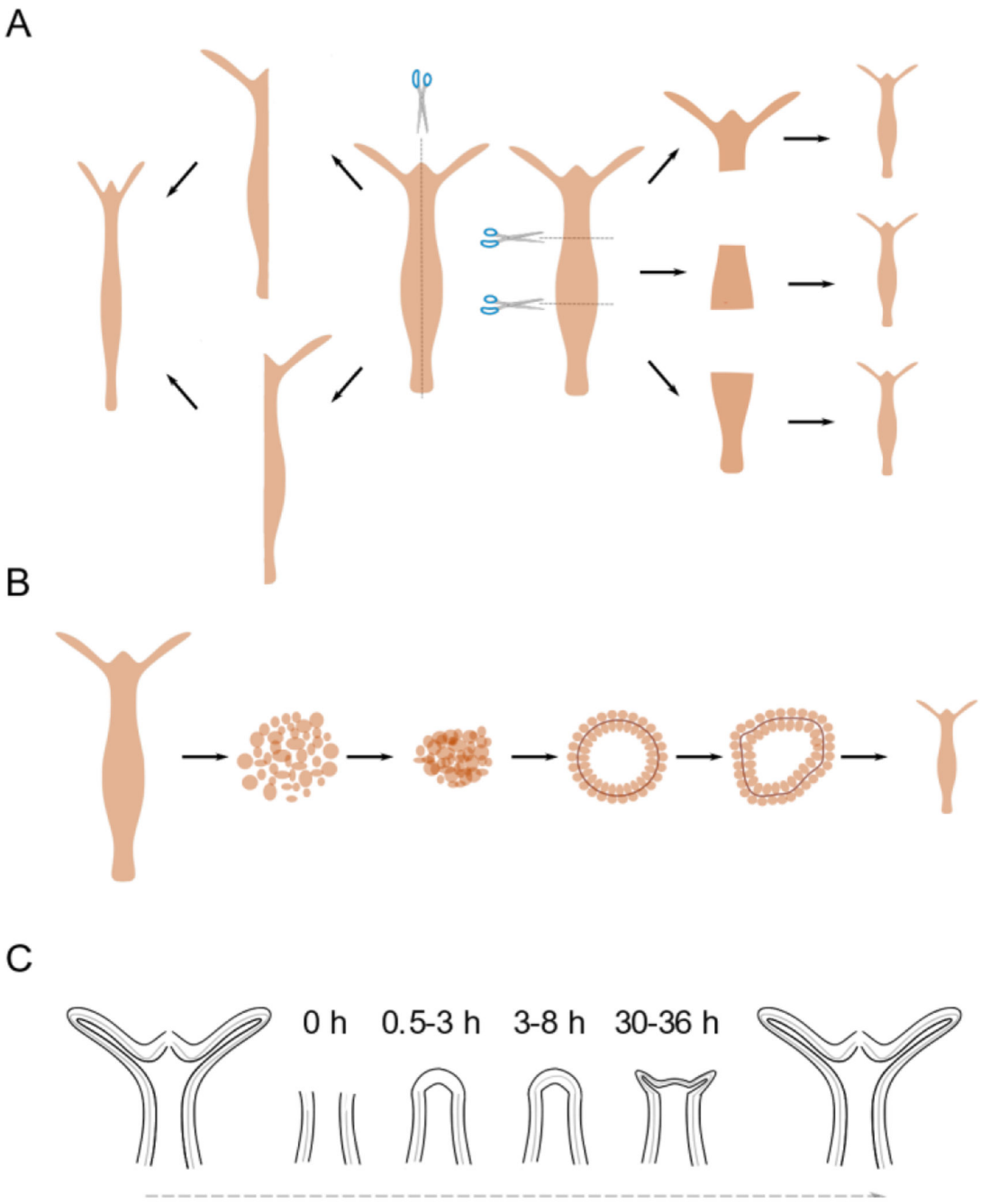


**Figure 2. Regeneration capacities of *Hydra vulgaris*.**

The ability of *Hydra* to regenerate when dissected in various manners and the morphological changes observed are shown. A) *Hydra* can regenerate missing parts upon both transverse and longitudinal dissection. B) Regeneration from dissociated and reaggregated cells. C) Kinetics of gross morphological changes during *Hydra* head regeneration.

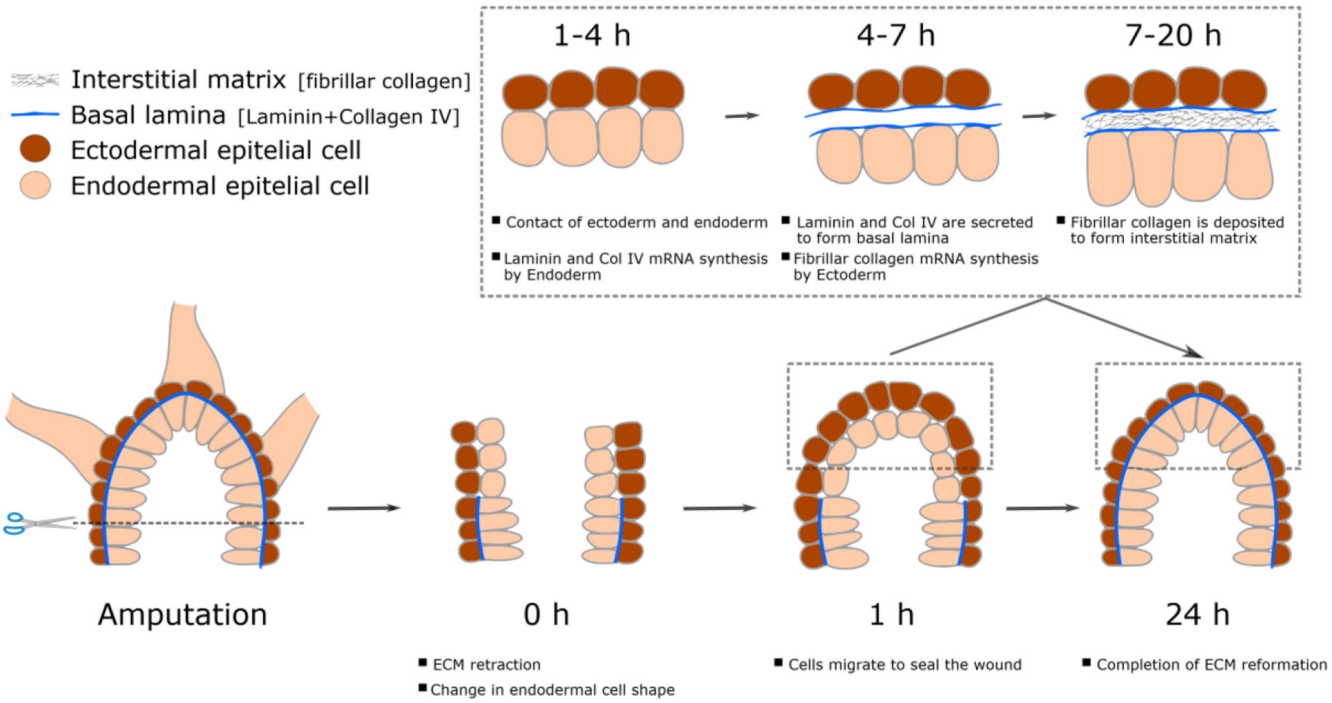


**Figure 3. Cellular and structural changes during head regeneration in *Hydra***

ECM is retracted immediately upon decapitation followed by change in endodermal cell shape. This is succeeded by wound closure and secretion of ECM components completing the early stages of head regeneration.




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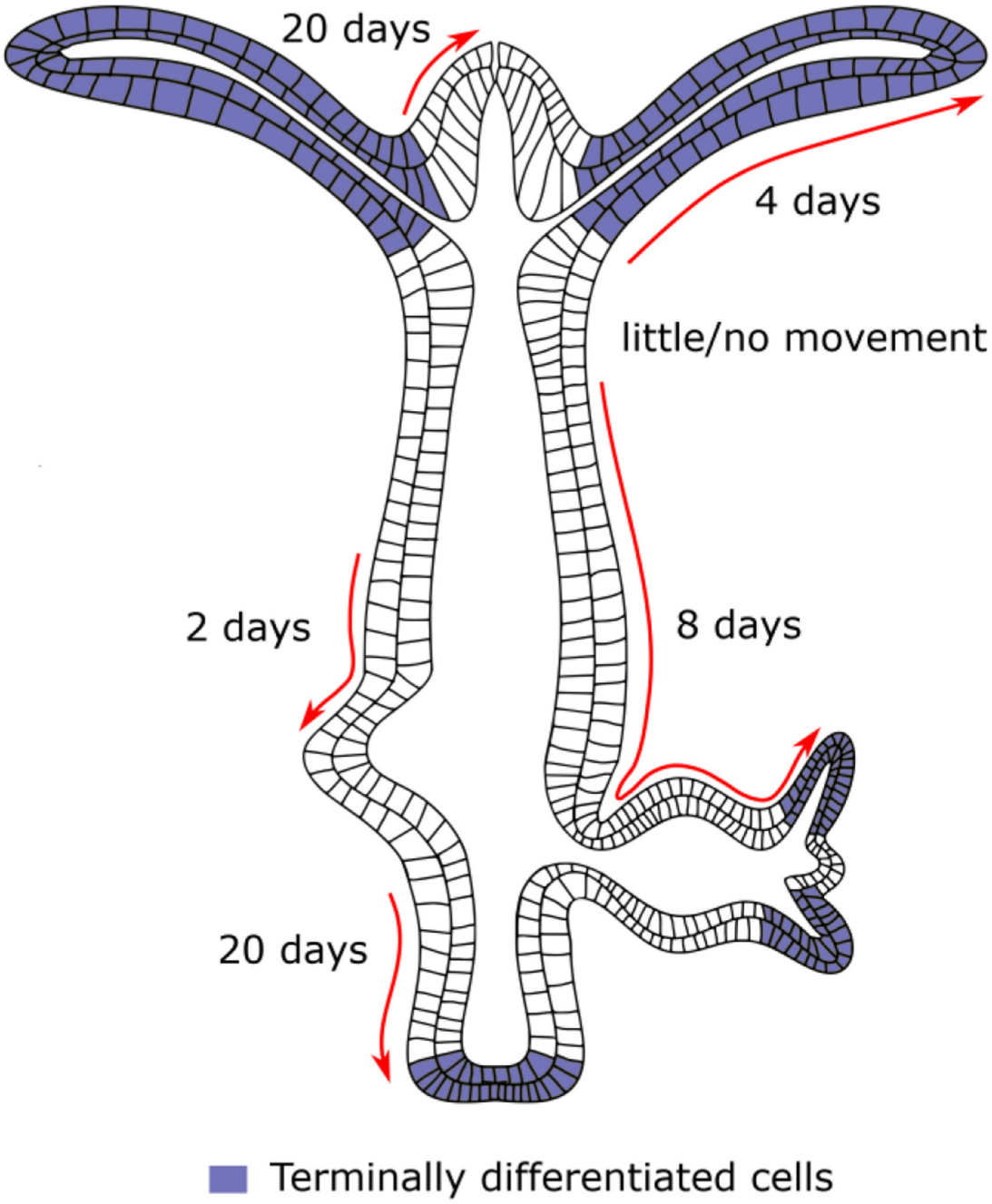


**Figure 4. Cellular dynamics in *Hydra***

The different cell movements that take place during maintenance of the *Hydra* body plan are depicted here. Cells undergo continuous proliferation and movement in both directions along the oral-aboral axis. The empty cells denote proliferative cells and the blue coloured cells are terminally differentiated cells. The number of days taken for cells to reach their respective destinations such as buds or the hypostome and peduncle termini are shown. The arrows depict the direction of cell movement.

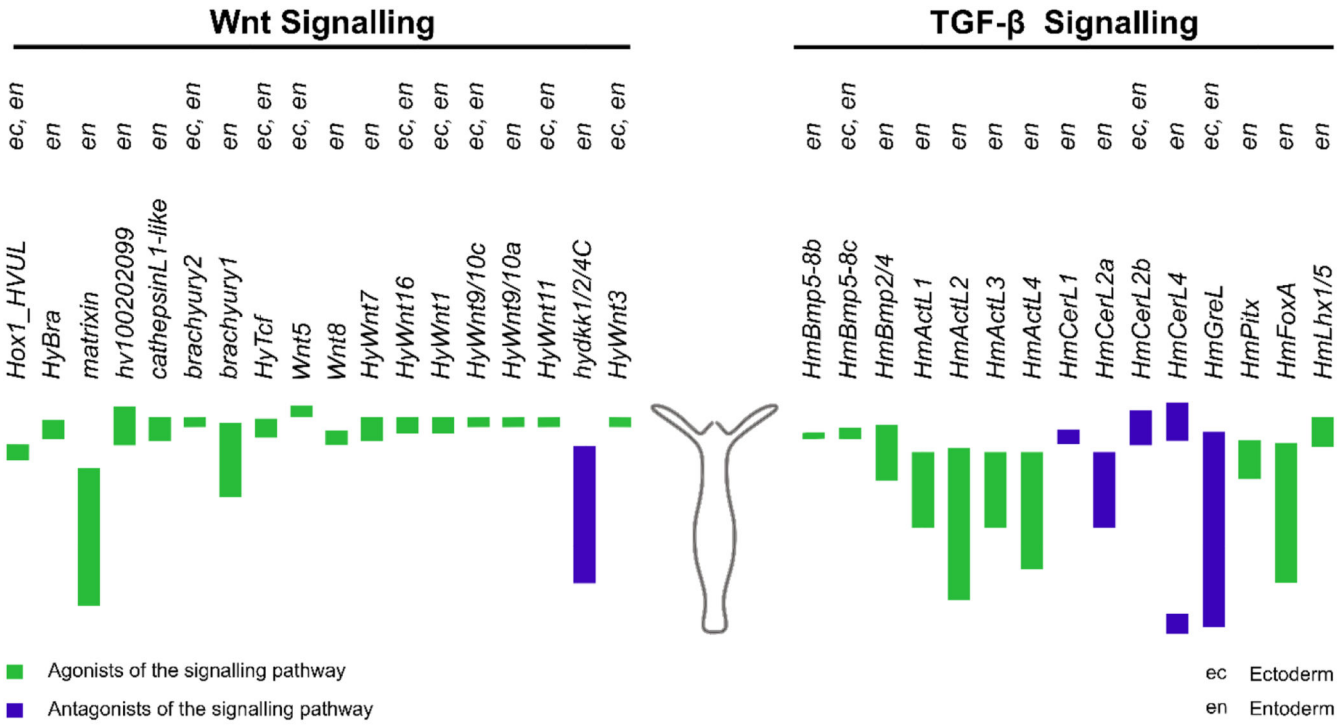


**Figure 5. Expression profile of Wnt and TGF-beta superfamily signalling components along the *Hydra* body axis**

Molecular components that have been shown to be regulated by Wnt (left) and TGF-β (right) signalling pathways have been depicted here. The coloured bars represent the localisation of gene expression along the body column of the *Hydra* polyp. Green bars represent molecules that play a positive regulatory role and the blue bars represent negative regulators of the pathways. The expression of the genes in the ectoderm and endoderm of *Hydra* has been denoted as *ec* and *en* respectively.




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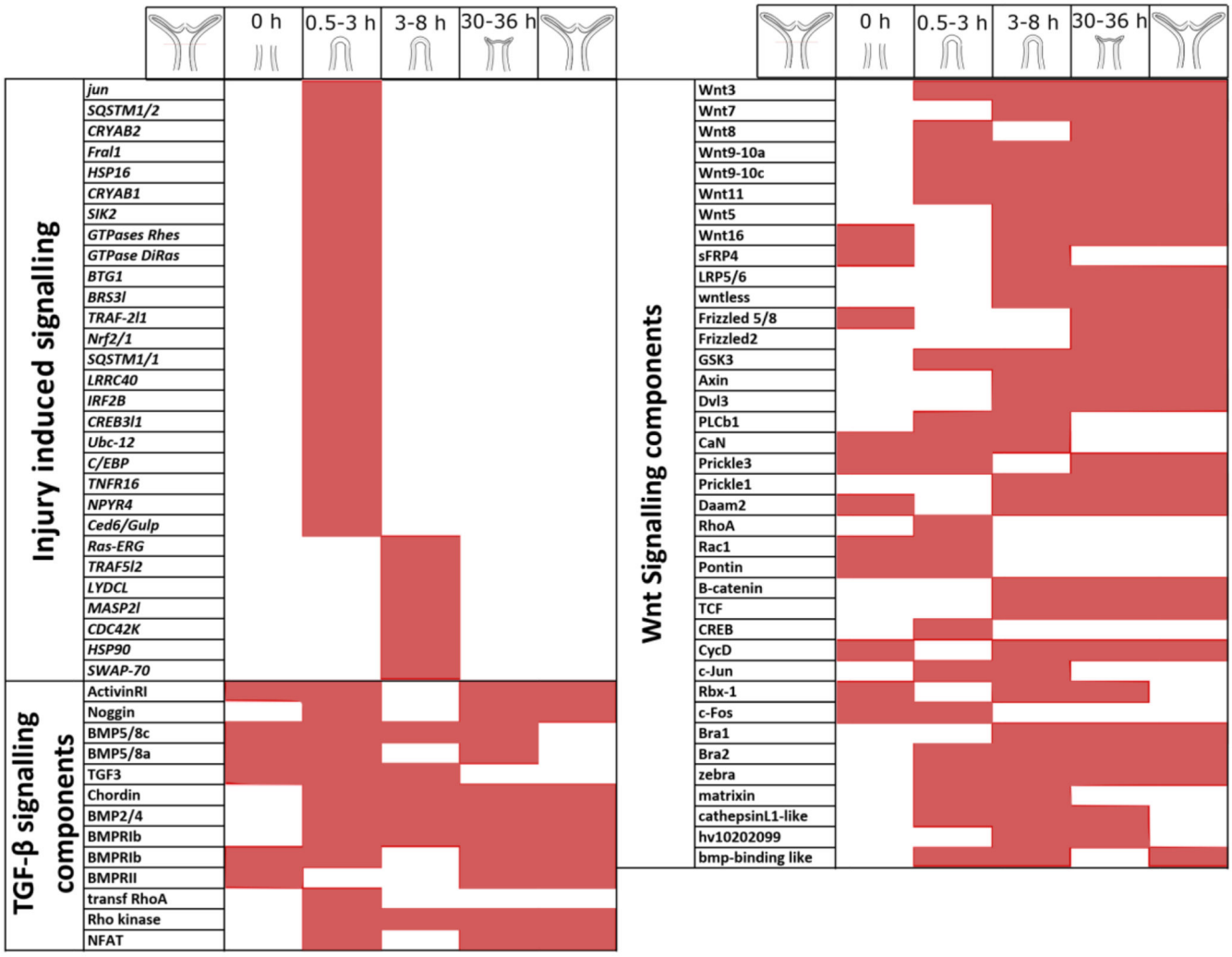


**Figure 6. Dynamic expression of signalling molecules during *Hydra* head regeneration**

Components of multiple signalling pathways that are reported to be differentially regulated across the regeneration time course have been depicted. The signalling pathways shown are Injury responses, TGF-β signalling pathway members and Wnt signalling pathway members. The regeneration timeline is depicted above the table. The shaded region for each gene denotes the time when mRNA expression of the respective molecule has been recorded.




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## Table 1

**Animals with diverse ability to regenerate complex tissues across the animal phyla**

| NON-CHORDATES | Sub-clade | Animal | Whole-organism | body part/Structure |
|---|---|---|---|---|
| **Porifera** | Homoscleromorpha | *Oscarella lobularis* | yes | |
| | Demospongiae | *Halisarca dujardini* | yes | |
| | Calcaria | *Sycon cilatum* | yes | |
| | | *Leucosolenia complicata* | yes | |
| **Ctenophora** | Tentaculates | *Mnemiopsis leidyi* | yes | |
| | | *Vallicula multiformis* | yes | |
| **Cnidaria** | Hydrozoa | *Hydra* | yes | |
| | | *clytia hemispherica* | no | partial medusa |
| | Anthozoa | *Nematostella* | yes | |
| | Scyphozoa | *Aurelia aurita* | only juvenile medusa | |
| | Cubozoa | *Carybdea marsupialis* | polyp stage | |
| **Platyhelminthes** | Trematoda | *Macrostomum lignano* | yes | |
| | | *Monocelis* | yes | |
| | | *Schmidtea mediterranea* | yes | |
| | | *Dugesia japonica* | yes | |
| | | *Girardia tigrina* | yes | |
| **Arthropoda** | | | | |
| Crustacea | Decapoda | *Menippe mercenaria* | no | chela |
| Chelicerata | Arachnids | *Latrodectus variolus* | no | limb |
| Hexapoda | Insecta | *Gryllus bimaculatus* | no | limb |
| Hexapoda | Insecta | *Tribolium castaneum* | no | limb |
| Hexapoda | Insecta | *Drosphila* | no | wing imaginal discs of larva |
| **Annelida** | Oligochaeta | *Pristina leidy* | yes | |
| | | *Lumbriculus sp* | yes | |
| | Polychaeta | *Chaetopterus variopedatus* | yes | |
| | | *Myxicola aesthetica* | yes | |
| **Mollusca** | Cephalopoda | *Sepia officinalis* | no | limb |
| | | *Loligo pealei* | no | limb |
| | | *Argonauta* | no | limb |
| | | *Octopus* sp. | no | limb |
| | Bivalvia | *Mesodesma mactroides* | no | inhalent siphon |
| **Echinodermata** | Asteroidea | *Leptasterias hexactis* | yes | |
| | | *Asterias rubens* | yes | |
| | | *Coscinasterias muricata* | yes | |
| | | *Asterina gibbosa* | no | arm |
| | Ophiuroidea | *Amphiura filiformis* | no | arm, pyloric caeca and cardiac stomach |

| NON-CHORDATES | Sub-clade | Animal | Whole-organism | body part/Structure |
|---|---|---|---|---|
| | Crinoidea | *Antedon mediterranea* | no | arm |
| | Holothuroidea | *Holothuria glaberrima* | no | appedages and visceral organs |
| | Echinoidea | *Paracentrotus lividus* | no | external appendages |
| | | *Lytechinus variegates* | larva | |
| | | *Strongylocentrotus purpuratus* | larva | |
| **HEMICHORDATA** | | | | |
| | Enteropneusta | *Ptychodera flava* | no | proboscis |
| **CHORDATA** | | | | |
| Tunicata | Ascidiacea | *Botrylloides violaceus* | yes | |
| | Ascidiacea | *Ciona intestinalis* | no | oral siphon, branchial sac and pharynx |
| Cephalochordata | Leptocardii | *Branchiostoma lanceolatum* | posterior and anterior body parts | |
| Pisces | Actinopterygii | *Danio rerio* | no | heart, lens, fins |
| | | *Sternopygus macrurus* | no | tail |
| Amphibia | Urodela | *Notophthalmus viridescens* | | limb, tail, spinal cord, lens, jaw and apex of heart |
| | | *Cynops pyrroghaster* | | limb, tail, spinal cord, lens, jaw and apex of heart |
| | | *Ambystoma mexicanum* | | limb, tail, spinal cord, lens, jaw and apex of heart |
| | Anura | *Xenopus laevis* | | juvenile limb |
| Reptilia | | | | |
| | Squmata | *Anoles caolinesis* | no | tail |
| Mammals | | *Dama dama* | no | antlers |
| | | *Cervus sp.* | no | antlers |
| | | *Rangifer tarandus* | no | antlers |
| | | *Mus musculus* | no | digit tips in fetal and juvenile stages |
| | | *Homo sapiens* | no | finger tips of children |