

1 [Development, Growth & Differentiation: Review]

2
3 Regeneration in the Enteropneust Hemichordate, *Ptychodera flava*, and Its
4 Evolutionary Implications

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17
18 Running title: Hemichordate Regeneration

19

1 Abstract:

2 Hemichordates are marine invertebrates that are closely related to chordates, but while
3 their body plans are comparable to those of chordates, they possess a remarkable
4 capacity for regeneration, even as adults. A small fragment is sufficient to form a
5 complete individual. Unlike echinoderms, their larvae transform directly into adults;
6 therefore, hemichordate systems offer clear morphological and molecular parallels
7 between regeneration and development. Morphological events in regeneration are
8 generally similar to organogenesis in juveniles. Nonetheless, comparative analysis of
9 gene expression in these two morphological phenomena suggests that hemichordate
10 regeneration is regulated by regeneration-specific mechanisms, as well as by
11 developmental mechanisms. Dependency upon resident pluripotent/multipotent stem
12 cells is a significant difference in metazoan regeneration, and such stem cells are
13 essential for regeneration in many lineages. Based on the present gene expression study,
14 regeneration in acorn worms is more closely related to that in vertebrates, because it
15 employs endogenous stem cell-independent transdifferentiation.

16

17 (150/250 words)

18 Keywords: evolution, hemichordate, pluripotency, regeneration, transdifferentiation

19

20 Introduction:

21 Hemichordates, a phylum within the Deuterostomia, are marine invertebrates.
22 Echinoderms, including sea cucumbers and sea stars, are commonly used as model
23 systems for studying invertebrate deuterostome regeneration. While echinoderms lose
24 their bilateral body plans as adults, hemichordates maintain bilateral body plans
25 throughout their life cycles. This trait is reasonable for comparing regenerative
26 capabilities based on body plan and/or cell-type complexity among bilaterian lineages.
27 Some acorn worms with high regenerative capacity can regenerate missing parts within
28 two weeks. Recently, genomes of acorn worm taxa exhibiting direct and indirect
29 development were decoded and massive cDNA libraries were constructed. In addition,
30 molecular analytical techniques (e.g. *in situ* hybridization and knock-down analysis) are
31 being developed to establish platforms for understanding molecular mechanisms of
32 hemichordate regeneration. In this review, we summarize classic reports and modern
33 molecular studies on hemichordate regeneration and compare mechanisms underlying

1 regeneration among bilaterians.

3 **Overview of hemichordates and regeneration**

4 Hemichordates are marine invertebrate deuterostomes, a sister group to
5 echinoderms. They exhibit bilateral body plans through their life cycles. The phylum
6 Hemichordata consists of two major groups, enteropneust acorn worms and
7 pterobranchs, which have very different morphologies. Acorn worms attain adult sizes
8 ranging from several millimeters to two meters and live on the seafloor, including the
9 intertidal zone and deep seas (Worsaae *et al.* 2012; Cannon *et al.* 2013). Pterobranchs
10 are tiny animals and their known habitats are very limited. The number of classified
11 pterobranch species is limited due to the difficulty of finding them (Tassia *et al.* 2016).
12 Bulk gene analyses based on whole genome sequences suggest that extant
13 deuterostomes comprise three major groups: chordates, hemichordates, and
14 echinoderms. Morphological characters of the common ancestor of deuterostomes are
15 hypothesized to resemble those of extant acorn worms (Simakov *et al.* 2015; Cannon *et*
16 *al.* 2016; Rouse *et al.* 2016).

17 Two different developmental patterns, i.e. direct and indirect development, are
18 observed in acorn worm embryogenesis (Fig. 1A). Planktonic larvae of indirect
19 developers, known as tornaria, have typical dipleurula-type morphology (Garstang
20 1928). Tornaria larvae do not form any adult rudiments during metamorphosis because
21 larval structures transform into adult forms (Agassiz 1873). Direct developers become
22 juveniles with complete sets of adult structures after undergoing embryogenesis that
23 resembles larval metamorphosis of indirect developers (Burdon-Jones 1952).

24 Adult enteropneusts are vermiform animals. Most species do not have
25 prominent structures (e.g. appendix); however, during the reproductive season, gonads
26 of species belonging to the family Ptychoderidae swell and become extended lobes
27 called genital wings. The bodies of adult worms are divided into three regions: an
28 anterior proboscis (protosome), a middle collar (mesosome), and a trunk (metasome)
29 (Fig. 1B). The mouth is located on the ventral side of the anterior region of the collar.
30 The digestive tract passes straight through the trunk and the anus is located at the
31 posterior end of the body.

32 Hemichordates display several features that may be comparable to specific
33 chordate attributes, such as gill slits on the dorsal side of the anterior trunk. In addition,

1 a stomochord in the proboscis, a notochord, and a dorsal nerve cord forming a hollow
2 tube in the mesosome, imply a close relationship between hemichordates and chordates.
3 Although structural homologies of these organs are still controversial (Satoh *et al.* 2014;
4 Tagawa 2016), formation of gill slits and pharyngeal arches in deuterostomes is
5 regulated by an orthologous gene cluster (Ogasawara *et al.* 1999; Gillis *et al.* 2012;
6 Simakov *et al.* 2015).

7 Bodies of acorn worms are covered with characteristic aromatic mucus,
8 containing phenol bromide and/or indole bromide (Ashworth & Cormier 1967; Higa &
9 Scheuer 1977). The body is easily broken off by friction or shearing, but missing parts
10 are readily regenerated. Regenerative capacity varies, depending on the species, and
11 some hemichordates can regenerate complete individuals from fragments. Moreover,
12 there are species that reproduce asexually through regeneration (Gilchrist 1923; Packard
13 1968; Miyamoto & Saito 2010). Even fragments that lack the central nervous system or
14 pharyngeal gill slits can regenerate intact animals. In these regards, hemichordates differ
15 greatly from chordates, in which regeneration is strictly limited.

16

17 **Morphological characters of hemichordate regeneration**

18 The first described hemichordate was *Ptychodera flava* (Eschscholtz 1825), a
19 species that we have been studying; however, it was originally thought to be a sea
20 cucumber. Later Kowalevsky (1866) identified gill slits in acorn worms, and
21 Metschnikoff described hemichordate developmental stages (1869, 1870). Bateson
22 (1885) proposed that the Hemichordata be classified as a subphylum of the Chordata,
23 based on morphological and developmental characters, but later the subphylum
24 Hemichordata was elevated to the status of a phylum, where it remains.

25 The most distinctive ability exhibited by acorn worms is the reconstruction of
26 intact individuals from fragments that result from cutting at an arbitrary position along
27 the anterior-posterior (AP) axis. Unlike planarians, there are no reports that
28 hemichordates can regenerate from small cell masses, but this ability is frequently used
29 to regenerate themselves after injury and reproduce new individuals. Intertidal species
30 may depend on frequent regeneration. For example, in a population at Oahu, Hawaii,
31 about 2% of collected specimens were regenerating or had recovered from injury within
32 the preceding several months (Humphreys *et al.* 2010). Furthermore, even species
33 known to employ sexual reproduction show biased sex ratios in several habitats (Rao

1 1954; Ritter & Davis 1904). These observations suggest that some populations may
2 maintain their populations by asexual reproduction through fragmentation and
3 regeneration. Another mode of asexual reproduction has also been reported, in which
4 fragments called regenerands are produced by autotomy and each regenerand regrows
5 into a new individual (Gilchrist 1923; Packard 1968; Miyamoto & Saito 2010). In wild
6 specimens, regeneration sites can often be identified based on different pigmentation of
7 newly formed tissue. The frequency of such observations suggests that most
8 regeneration in nature is due to injury.

9 The degree of regenerative capability also varies widely among acorn worms.
10 For example, *Glossobalanus minutus* can only regenerate a lost anterior part, and
11 reproduction does not occur if the posterior part is lost (Dawydoff, 1909). In a
12 regeneration study of *Saccoglossus kowalevskii*, only the posterior part could regenerate
13 a missing proboscis and collar when it was cut at the collar region, while only the
14 anterior could regenerate when it was cut in the center of the trunk (Tweedel, 1961).
15 This means that in *S. kowalevskii*, the direction of regeneration depends upon the
16 location of the injury.

17 In contrast, our model organism, *Ptychodera flava*, can regenerate in both
18 directions, irrespective of amputation site, when we used worms immediately after
19 collection. In this process, original structures are restored sequentially starting with the
20 most distal end of the missing part (Fig. 2). This type of regeneration fits the model
21 called “distalization and intercalation” (Agata *et al.* 2007). The success of regeneration
22 can exceed 90% or, but extremely rarely, individuals with multiple proboscises and/or
23 collars are seen (Fig. 3A). The frequency of errant regeneration in nature is estimated at
24 <0.03% (Nishikawa, 1985), but can be increased by surgically perturbing the cut surface
25 (unpublished data). This suggests that some factors governing axis polarity and/or
26 patterning at the site of the injury affect regeneration (Fig. 3B).

27 28 **Molecular mechanisms of acorn worm regeneration and their association with** 29 **development**

30 Molecular biological techniques, such as *in situ* hybridization, have been
31 applied to various non-model organisms in recent years and comparative studies have
32 yielded numerous important insights. *Ptychodera flava* is the first hemichordate to be
33 studied not only in relation to development, using molecular biology techniques, but

1 also with respect to regeneration, because of its remarkable capabilities. Several species
2 of both direct and indirect developers, including *P. flava*, have been utilized for
3 comparative developmental analyses. Studies of axial patterning in embryogenesis of a
4 direct developer, *S. kowalevskii*, found ectodermal AP patterning that is conserved
5 among hemichordates and vertebrates (Lowe *et al.* 2003; Aronowicz & Lowe 2006;
6 Pani *et al.* 2012; Darras *et al.* 2018). Larval and adult body plans of an indirect
7 developer, *Schizocardium californicum*, have been compared by analyzing over 20
8 transcription factors involved in ectodermal AP patterning (Gonzalez *et al.* 2017). The
9 results suggest that the Hox and Wnt gene families are important in patterning the AP
10 axis during hemichordate development, as in most bilaterians. Expression patterns of
11 these genes, particularly *hox* genes, during trunk formation of juveniles, are clearly
12 conserved between direct and indirect developers (Fig. 3B).

13 Whether these axial patterning genes show similar expression patterns during
14 regeneration is still unclear; however, the comparison of *hedgehog* gene expression
15 patterns between regeneration and embryogenesis in *P. flava* suggests that there may be
16 regeneration-specific mechanisms for specifying positional information (Arimoto &
17 Tagawa 2015). *Hedgehog* is known as a marker that is expressed at the anterior end of
18 larvae during bilaterian metamorphosis, and it is also expressed in the pharyngeal region
19 from late metamorphosis into the young juvenile stage (Pani *et al.* 2012; Miyamoto &
20 Wada 2013). It is reported that the anterior expression of *hedgehog* disappears in
21 juvenile *Balanoglossus simodensis*, which is closely related to *P. flava* (Miyamoto &
22 Wada 2013). Therefore, transient anterior expression of *hedgehog* might be expected in
23 regeneration, if molecular mechanisms that form adult anterior structures during
24 metamorphosis are simply reused. However, in expression analysis using *in situ*
25 hybridization, *hedgehog* expression was confirmed only in the pharynx and was not
26 detected at the anterior tip during regeneration (Arimoto & Tagawa 2015). The absence
27 of *hedgehog* expression at the anterior tip of the blastema supports the idea that the
28 regenerative rudiment and anterior structures in metamorphic larvae have different
29 natures. These results also imply that the blastema is not formed by simply repeating the
30 metamorphic process. Assuming that, *P. flava* has at least two types of mechanisms, i.e.
31 adapting metamorphosis or regeneration, to form adult anterior structures.

32 A major unresolved question in acorn worm regeneration concerns how cells
33 in the regenerating region are supplied. A previous study using *P. flava* suggested that

1 mesenchymal cells contribute to some of the newly formed tissues (Rychel & Swalla
2 2008). These cells migrated to the injured site soon after amputation and proliferating
3 cells became restricted to the anterior regenerating end. This process was followed by
4 apoptosis and then rudiments of missing structures started to form. We visualized cell
5 division during regeneration of *P. flava* by labeling with EdU (an analog of thymidine)
6 and found that cell division is highly activated in the dorsal region of the regeneration
7 site (unpublished data). These results imply that the location of the blastema is
8 determined by the dorsal nerve cord; thus, hemichordate regeneration may be
9 nerve-dependent, as in several other groups of animals. Further analysis is required to
10 determine whether migrating cells are themselves pluripotent or whether dividing cells
11 near the wound site are derived from migratory cells.

12 We conducted an analysis of comprehensive gene expression in *P. flava*
13 regeneration. We compared ESTs from six early developmental stages and four adult
14 tissues and found that gene expression profiles of regenerating tissues tend to be
15 down-regulated compared to other EST libraries (Tagawa *et al.* 2014; unpublished data).
16 Luttell *et al.* (2016) also reported gene expression profiles using RNA-seq in early
17 regenerative stages, showing that potential homologs of vertebrate somatic cell
18 reprogramming factors, such as *pou*, *klf*, and *sox*, are expressed in a stage-specific
19 manner during regeneration. These potential homologs of hemichordate and vertebrate
20 reprogramming factors are classified into closely related gene families; nevertheless,
21 they do not show direct orthology. However, the flexibility of the combination of
22 vertebrate reprogramming factors (Brouwer *et al.* 2016) suggests that these homologs
23 possibly regulate somatic cell reprogramming in hemichordates. The next-generation
24 sequencing data is consistent with our results of gene expression patterns from *in situ*
25 hybridization (unpublished data). Together, these results indicate that re-acquiring a
26 pluripotent state may be a key to regeneration in hemichordates.

27

28 **Comparison of regulatory mechanisms of regeneration among bilaterians**

29 The blastema is formed during animal regeneration and can easily be
30 distinguished morphologically and histologically from differentiated cells. In anterior
31 regeneration of *P. flava*, the blastema is formed on the anterior surface of the
32 wound-repair surface and missing structures are reformed sequentially from the distal
33 end of the proboscis, collar, or trunk, starting from the blastema (Willey 1898; Fig. 2).

1 Furthermore, in the field, many individuals are seen undergoing regeneration and
2 sexually mature individuals without gonads are rarely encountered (Humphreys et al.
3 2010). From these observations, it is assumed that gonads can be regenerated even if
4 reproductive organs are completely lost, that is to say, all cell types including totipotent
5 germ cells can be recovered by regeneration.

6 Cells involved in acorn worm regeneration seem to be pluripotent; however,
7 the source of these pluripotent cells is unknown. There are two possible origins (Fig.
8 4A). One is that such stem cells are maintained in the adult body, as in planarians, and
9 that they give rise to the entire range of cell types and organs. The other is that
10 differentiated cells achieve pluripotency or multipotency through de-differentiation, as
11 in vertebrate regeneration. Our current findings, including gene expression studies in *P.*
12 *flava* described above, support the latter (Fig. 4B).

13 Body axis patterning in acorn worms is determined in early embryogenesis in
14 both direct and indirect developers, and body axes do not change before or after
15 metamorphosis, unlike in echinoderms. In a previous study (Arimoto & Tagawa 2015),
16 we proposed that anterior regeneration might be driven by regeneration-specific
17 mechanisms, different from those employed in embryogenesis. Are similar mechanisms
18 used for reconstruction of body axes and organ position in regeneration?

19 We have shown that a secondary AP axis can be induced anomalously by
20 surgery, as mentioned earlier. On the other hand, modification of dorsal-ventral (DV)
21 axis patterning in regeneration has not been reported. DV axis determination in acorn
22 worm embryogenesis is regulated by highly conserved mechanisms in bilaterians, e.g.
23 antagonism of BMP and Chordin which are known as a dorsalizing and a ventralizing
24 factor in non-chordates, respectively, as well as AP axis determination (Lowe *et al.*
25 2006; Röttinger & Martindale 2011). However, since surgery does not induce DV axis
26 remodeling during regeneration, positional information in the DV axis may not be
27 reconstructed during regeneration, but may reflect information existing in the trunk. It is
28 reported that muscles are essential to reconstruction of body axis information in
29 planarian regeneration (Witchley *et al.* 2013); similar mechanisms may also exist in
30 acorn worms.

31 *Hedgehog* genes, described above, also function throughout the body during
32 protostome regeneration. In planarians, the most well-understood regeneration model
33 system, *hedgehog* is involved in determination of the AP axis in opposition to *wnt*

1 signals (Rink *et al.* 2009; Yazawa *et al.* 2009). In polychaete annelids, *hedgehog*
2 functions as a segment polarity gene when regenerating lost somites (Niwa *et al.* 2013).
3 The expression pattern of *hedgehog* observed in *P. flava* regeneration is different from
4 the wnt/hedgehog antagonism model in regeneration in the aforementioned protostomes
5 (Arimoto & Tagawa 2015). Therefore, molecular mechanisms for body axis
6 determination and/or position information in acorn worm regeneration differ from those
7 of protostomes.

8 In deuterostome regeneration, adults with true bilateral body plans are
9 extremely vulnerable to bisection transverse to the AP axis. Separation of the gill and
10 anus regions is invariably fatal, even in non-vertebrate chordates (Somorjai *et al.* 2012;
11 Fig. 4A). Since such tendencies also exist in some species of acorn worms,
12 comparison of molecular responses among hemichordates with different regenerative
13 abilities might reveal the evolutionary history of susceptibility to AP axis amputation.

14 15 **New technologies accelerating studies of hemichordate regeneration**

16 To understand molecular mechanisms of regeneration in hemichordates, it is
17 necessary to develop optimized experimental systems for functional analysis for the
18 model animals being investigated. In acorn worm embryogenesis, it is possible to
19 inhibit signaling pathways and then to induce abnormal morphogenesis using inhibitors
20 commonly used for other marine invertebrates (Darras *et al.* 2011). Although there are
21 no reports of treating hemichordates with such inhibitors during regeneration, in
22 principle, this could be done to see whether they have similar effects to those observed
23 during embryonic development. Skillful microinjection using short interfering RNAs or
24 recombinant proteins has been employed to study early developmental embryos of *S.*
25 *kowalevskii* (Lowe *et al.* 2006). This system provided gain- or loss-of-function analysis
26 with high target specificity. Such targeted functional assay systems for regenerating
27 individuals have not been established yet, but gene transfer into regenerating tissues by
28 electroporation may be a promising technique for regeneration analysis.

29 Identifying the source and the capacity for differentiation of regenerating cells
30 are the two most important points necessary to understand mechanisms of regeneration
31 in hemichordates. As a first step, it is necessary to analyze cell lineages of regenerative
32 tissues and then to grasp the restriction of differentiation potential of those cells
33 involved in regeneration. Currently use of the CRISPR/Cas system to create

1 knock-out/knock-in animals, might be one of the most powerful methods (Sasaki *et al.*
2 2014; Lin & Su 2016). In addition, single-cell analysis using next-generation
3 sequencing, e.g. pseudo-time course analysis, would help to identify the sources of cells
4 in regenerated structures (Guo *et al.* 2017). It is also desirable to obtain more
5 comprehensive RNA-seq data, to increase sampling frequency, and to cover the major
6 morphogenetic events during regeneration. Comparisons of comprehensive RNA-seq
7 data among various animals with high temporal resolution could illuminate mechanisms
8 underlying limitations of regeneration in each animal.

10 **Conclusions:**

11 The specificity of hemichordate regenerative capability enables
12 hemichordates to reconstruct intact individuals from fragments, even when the AP axis
13 is completely cut at any given position. Generally, such damage is lethal to bilateral
14 adult deuterostomes, such as chordates. The high regenerative ability of *P. flava* is
15 thought to be driven not only by the same mechanisms as in embryogenesis, but also by
16 regeneration-specific mechanisms that directly specify the structure of adult organs.

17 Whether hemichordate regeneration depends on resident pluripotent stem
18 cells as in planarian regeneration, is open to challenge. Based on our current gene
19 expression study of reprogramming factors using *in situ* hybridization and RNA-seq
20 analyses, however, acorn worm regeneration is more closely related to that of
21 vertebrates, occurring by transdifferentiation. It is interesting to consider the
22 evolutionary change in regenerative cell source from resident pluripotent stem cells
23 found in many metazoan lineages to de-differentiated stem cells, characteristic of
24 chordate lineages.

25 Comparative analysis among species and/or higher taxa is expected to yield
26 insights into evolutionary changes in regenerative capability, as well as genetic factors
27 that limit regenerative flexibility among deuterostomes. Such knowledge may help to
28 enhance regenerative capability even in species with limited regenerative ability, such
29 as humans.

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1 **Figure legends:**

2

3 Figure 1. (A) A lifecycle of hemichordates. Gray and green arrows indicate
 4 developmental and regenerative processes, respectively. Indirect developers, such as
 5 *Ptychodera flava*, make tornaria larvae and the larvae transform into vermiform
 6 juveniles. Direct developers do not have larval stages; thus, the juveniles are directly
 7 developed from the gastrulae. Fragments of adult worms can make complete individuals
 8 through regeneration. (B) A dorsolateral view of a mature female *Ptychodera flava*, an
 9 enteropneust hemichordate. The body surface is fully covered with colorless mucus.
 10 The yellowish mucus is secreted when the animal is exposed to physical stimuli. This
 11 mucus has a characteristic odor. The trunk, posterior to the collar is elastic and gonads
 12 of mature worms are elongated in the middle of the hepatic region where two lines of
 13 hepatic sacculae are aligned. At the dorsal midline between the anterior gonads, two
 14 lines of pharyngeal gill slits form the branchial region. The branchial region represents
 15 the dorsal side of the pharyngeal region so that gill slits are also called pharyngeal gills.
 16 Whole-body regeneration is elicited by a cut at an arbitrary position along the
 17 anterior-posterior axis. Through this process, more than one individual is reproduced.
 18 An arrowhead indicates the anterior tip of the proboscis. The inset shows a lateral view
 19 of anatomical scheme of anterior structures. a, anus; c, collar; dn, dorsal nerve cord; go,
 20 gonads (genital wings); gs, gill slits (pharyngeal gills); h, hepatic sacculae; m, mouth; pr,
 21 proboscis; s, stomochord.

22

23 Figure 2. Anterior regeneration of *Ptychodera flava*, from Humphreys et al. (2010). (A)
 24 Intact worm before amputation between the branchial and hepatic regions. (B) Posterior
 25 trunk just after amputation. (C) At two days post-amputation (dpa), the area around the
 26 wound is swelling and the hole is almost closed; however, the digestive tract is still
 27 open to the outside. (D) The wound repair process finishes at 3 dpa. A tiny shield
 28 blastema appears on the tip of the anterior end. (E) The blastema becomes visible to the
 29 naked eye at 4 dpa. This visible part becomes the proboscis. (F) At 5 dpa, two ridges of
 30 the collar rudiment are formed on both sides of the most prominent mass of the
 31 blastema. (G) Lateral ridges of the blastema completely cover the collar region at 7 dpa.
 32 The mouth opens at the ventral region of the regenerating proboscis. (H) Regeneration
 33 of functional proboscis and collar is complete at 12 dpa. The worm starts digging in the

1 sand with the recovered proboscis. (I) At 17 dpa, regeneration continues to form the
 2 missing branchial region. This process intercalates new tissues between the regenerated
 3 anterior structures and the original posterior trunk. (J) Anatomical schemes of anterior
 4 regeneration. In this figure, left is anterior and top is dorsal. Arrowheads indicate the
 5 position of blastema. A shaded part at 7 dpa corresponds to the regenerating collar. (K)
 6 Regenerative capability of each part of the body based on a previous report (Nishikawa
 7 1977). Blue bars correspond to regions that remain in initial fragments. “Yes” and “No”
 8 indicate observed regeneration succeeded or not in each direction, respectively.
 9 “Autotomy” means that cutting in the point can induce regenerative autotomy. “Partially”
 10 means that regenerated individuals showed abnormal morphologies of the structure
 11 which was recovered in the direction.

12

13 Figure 3. Expression patterns of body-axis patterning genes and axial patterning error
 14 during anterior regeneration. (A) A regenerating individual with a secondary AP axis
 15 anomaly induced by surgery. Black and white arrowheads indicate primary and
 16 secondary regenerating structures, respectively. The speed of regeneration of secondary
 17 structure is slower than that of primary structure. (B) Expression patterns of body-axis
 18 patterning genes conserved between direct and indirect developers during the juvenile
 19 stage. Various members of the HOX family, including NKL, PRD, SINE, and TALE, are
 20 associated with development of the proboscis and collar. Positional information of the
 21 trunk is mainly regulated by *hox* genes of the HOXL subclass. The dorsoventral axis is
 22 determined by antagonism of BMP-Chordin as in protostome bilaterians. Expression
 23 patterns are based on previous reports (Lowe *et al.* 2003; Aronowicz & Lowe 2006;
 24 Lowe *et al.* 2006; Röttinger & Martindale 2011; Gonzalez *et al.* 2017).

25

26 Figure 4. Regenerative capabilities and mechanisms in metazoan lineages. (A)
 27 Comparison of regenerative capabilities among solitary metazoans based on Lai and
 28 Aboobaker (2018). Whole-body regeneration is a common characteristic of metazoans;
 29 however, residential pluripotent/multipotent stem cells are required for the process in
 30 many phyla. In hemichordate regeneration, it seems that differentiation potential of
 31 somatic cells is recovered, as in chordate regeneration. In chordates, organ or tissue
 32 regeneration depends on stem cells, and large scale structural regeneration is driven by
 33 de-differentiation of somatic cells. Parentheses indicate that dependencies are supported

1 by indirect evidence. Question marks mean that dependency types have not been
2 identified. NA, not applicable. (B) A possible model of hemichordate regeneration
3 based on our present knowledge. Wound repair completes before regeneration starts in a
4 strict sense, i.e. before blastema formation. Pluripotency-associated genes are expressed
5 in parallel with the repair process. De-differentiated cells form a blastema in the first
6 step of regeneration. Missing tissue is reconstructed by differentiation of the blastema
7 with cooperation of unknown regeneration-specific and conserved developmental
8 mechanisms.