

## Regeneration and Asexual Reproduction in Annelids: Cells, Genes, and Evolution

R. P. Kostyuchenko, V. V. Kozin, and E. E. Kupriashova

St. Petersburg State University, Universitetskaya nab. 7/9, St. Petersburg, 199034 Russia

e-mail: r.kostyuchenko@spbu.ru

Received October 19, 2015

**Abstract**—A comparative study of regeneration and asexual reproduction across annelids is presented. The importance and universality of early stages of the restoration morphogeneses, which, however, vary in details, is shown. Here, we concern on the conserved elements of genetic regulatory programs involved in both processes, responsible for cell fate plasticity and rapid changes of following re-establishment of positional identity along the anterior-posterior axis.

**DOI:** 10.1134/S1062359016030067

### INTRODUCTION

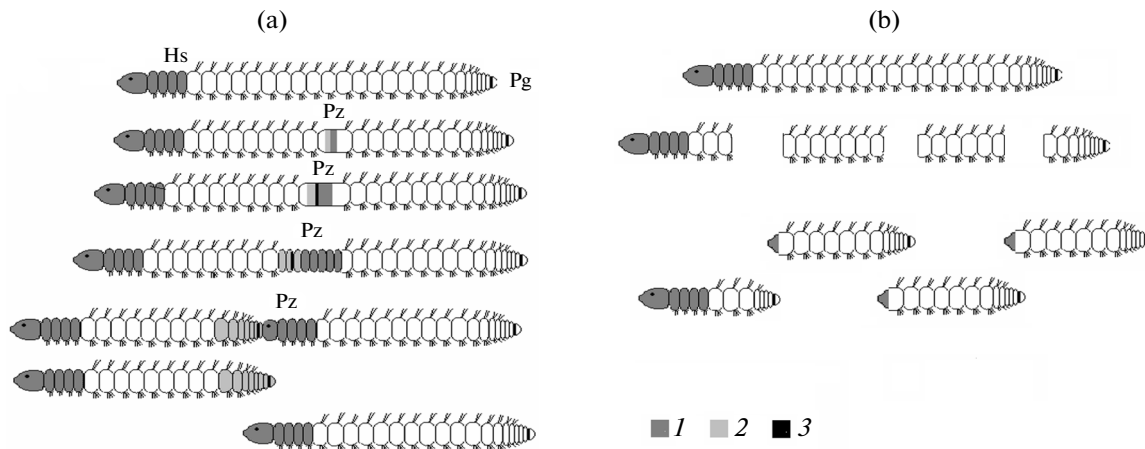
Development of animals is often associated with gametes followed by fertilization and cleavage, a series of mitotic divisions that give rise to a multicellular organism with differentiated organs and tissues. However, development persists after the termination of embryonic and larval stages. Growth, regeneration and asexual reproduction are post-embryonic developmental processes which are characteristic of a dramatic increase in the number of cells, determination of cell fate, terminal cell differentiation, body plan formation and the acquisition of functions. Nevertheless, regeneration and asexual reproduction are special types of development due to the ability to restore body parts and even an entire organism *de novo* agametically (Ivanova-Kazas, 1977; Stocum, 2012).

Regeneration is a fundamental feature found in animals of different organizational level. Cell turnover, the replacement of old cells with newly generated ones, as is the case in cell replacement of gut, is likely to be found in any multicellular organism. Such an outwardly invisible renewal occurring under normal physiological conditions is referred to as physiological regeneration. Reparative regeneration which is triggered by injury caused by accident, illness or surgery, proceeds in a more obvious fashion. Studies of exactly reparative regeneration are vigorously pursued as they can not only unveil fundamental mechanisms of cell differentiation and morphogeneses but also have prospects for practical applications (Stocum, 2012).

Although the regenerative abilities are widely spread among animal groups, they vary considerably among closely related species (Stocum, 2012). This feature of distribution of regenerative abilities across the phylum has not escaped the attention of the researchers: the minor differences serve as guides for uncovering the molecular underpinnings for initiation

and implementation of the process. For example, most planarians have unlimited capability of regeneration of head but some species demonstrate restriction or complete loss of this ability when cutting plane is moved to the pharynx region and to the posterior end of the body (Korotkova, 1997; Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013). With some molecular mechanisms of regeneration uncovered, the experiment to rescue full regeneration in *Dendrocoelum lacteum* and *Procoetyla fluviatilis* has been recently carried out through *Wnt*-pathway components manipulations. Head regeneration is normally either deficient or absent at some level along the anterior-posterior axis in these species (Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013). These new data on the reactivation of regenerative abilities in regeneration-deficient animals suggest the existence of some critical points responsible for gains and losses of the regenerative abilities. It also indicates a breakthrough in our understanding of this phenomenon in a particular animal model. Notably, flatworms are unique in having somatic stem cell population called neoblasts which is not present in other taxa. This limits extrapolation of acquired knowledge to other model organisms and highlights the importance of comparative approach to finding answers on key questions of regenerative biology.

Along with regeneration, asexual reproduction is also the kind of post-embryonic development in which entire body parts are formed *de novo* (Ivanova-Kazas, 1977; Korotkova, 1997). Asexual reproduction is common among Metazoa but it is unequally distributed across different phyla. Asexual reproduction is a usual stage of the life cycle or takes place as a response to some environmental conditions. This is common for many species and occasionally asexual reproduction is the only mean of reproduction for a species (Ivanova-



**Fig. 1.** Schematic view of asexual reproduction in annelids. (a) Paratomy, (b) architomy. (1) head region and cephalogenic part of fission zone, (2) somatogenic region of fission zone and its derivatives, (3) growth zone; Hs—head segments, Pz—paratomy zone, Pg—pygidium.

Kazas, 1977; Kharin et al., 2006). The forms of asexual reproduction are known to correlate with the complexity of the body plan and can be as diverse as animals are. However, the four main types of asexual reproduction by means of somatic cells are distinguished: fission, budding, cell aggregation, and polyembryony.

Asexual reproduction does not only have many similarities with post-traumatic regeneration but there is also correlation between the two processes. Species which reproduce asexually usually have prominent regenerative abilities (Morgan, 1901; Vorontsova and Liosner, 1960; Ivanova-Kazas, 1977; Korotkova, 1997). Lots of discussions caused by this observation concern the evolutionary relationship between regeneration and asexual reproduction. Most authors share the opinion that regenerative abilities are ancestral to asexual reproduction (Morgan, 1901; Vorontsova and Liosner, 1960; Korotkova, 1997; Bely and Wray, 2001). The opposing point of view is that regeneration arose from asexual reproduction (Sánchez Alvarado, 2000). The third opinion suggests that both kinds of post-embryonic development are equivalent (Berrill, 1952; Gibson and Paterson, 2003). Nevertheless, regeneration and asexual reproduction differ (Bely and Sikes, 2010), and studying of the differences can shed light on the fundamental mechanisms of initiation of the regenerative events and cell fate determination. The in-depth comparison between the two processes seems to be fruitful for clarifying principles which govern the choice of the developmental trajectory and the evolution of underlying mechanisms. Due to the diversity of animals, it is unlikely that common regeneration mechanisms shared by all species will ever be identified. Thus, further studies are needed to compare species across a given phylum. In this article, data on molecular and cellular mechanisms of regeneration and asexual reproduction of annelids are reviewed

(annelids are a key group of invertebrates demonstrating conserved embryonic development and variable post-embryonic development at the same time).

#### REGENERATION AND ASEQUAL REPRODUCTION IN ANNELIDS

Annelids are a large and diverse group of invertebrates, with species capable of regenerating a new tail or head or both simultaneously. Certain species can regenerate a whole worm even from a tiny fragment (Berrill, 1952; Herlant-Meewis, 1964; Korotkova, 1997; Bely, 2006). In addition, most annelids reproduce asexually, mainly by architomy type of transverse fission (a worm splits into fragments which then restore missing heads and tails) or by paratomy (new heads and tails emerge before splitting) (Fig. 1). Modified forms of paratomy and architomy can be called by specific terms in different animal groups, for example ctenodrilization, stolonization, schizometamery, and so on. Correlation between abilities to reproduce agametically and to regenerate lost body parts is shown for annelids as well as for other phyla (Morgan, 1901; Vorontsova and Liosner, 1960; Bely, 1999; Kharin et al., 2006).

Despite annelid regeneration has been studied over a century (Randolph, 1892; Herlant-Meewis, 1964; Korotkova, 1997; Bely, 2006; Kozin and Kostyuchenko, 2015), the first insights into the molecular mechanisms of the process came to light only in the last decade. Published literature mostly concern the data on regeneration events at the cellular level of investigation. These data are undoubtedly of great importance, yet the conclusions call for confirmation with the use of modern techniques. Though, dynamic changes observed at the cellular and tissue levels are key for understanding the molecular basis of regeneration. They provide much better understanding of regenera-

tion capacity in annelids. Across from regeneration, asexual reproduction in annelids is poorer studied. Data on asexual reproduction of annelids are exceedingly limited and concern mainly anatomical aspect, but even in this case, the emphasis is on events at cellular and tissue level of investigation (Christensen, 1959; Ivanova-Kazas, 1977; Myohara et al., 1999; Kharin et al., 2006; Zattara and Bely, 2011, 2013; Babakhanova et al., 2012).

### CELLULAR AND TISSUE PROCESSES

During regeneration and transverse fission, the old tissues are of great and often critical importance since the earliest stages of these processes (table). Thus following the amputation, nearly all annelids studied were able to close the wound by rapid muscle contraction or gut prolapse (rarely) (Herlant-Meewis, 1964; Korotkova, 1997). This is characteristic even for the regeneration-deficient species (Bely, 2006; Bely and Sikes, 2010). Absence of such a reaction leads to the death of the animals (Iwanoff, 1928; Bely, 2006). Prevention of the body liquid loss is also efficient during asexual reproduction (Lesiuk and Drewes, 1999; Kawamoto et al., 2005; Kharin et al., 2006). Following autotomy or amputation, extensive cell migrations to the site of injury occur. Several cell types of migrating cells suggested to perform different functions are known. The major challenge of these cells identifying and homologizing is the difficulty of interpretation of the histological findings based on fixed material (Cornec et al., 1987; Korotkova, 1997).

Undoubtedly, phagocytes migrate to the wound site at the early stage of regeneration to protect the animals against infection and engulf damaged cells and debris. In addition, phagocytes, along with coelomocytes, from which they seem to originate, comprise cell mass that help wound sealing (Cornec et al., 1987; Korotkova, 1997). However, other various cell types participating in the immune defense in some annelids, for example, amoebocytes in *Lumbricillus*, hyolocytes and macrophages in *Eisenia* (Cornec et al., 1987), have been described, their homology still being discussed.

In several species, among the cells migrating to the wound, there is a particular group comprised of round cells with a high nuclear-cytoplasmic ratio indicating their un- or slightly-differentiated state. These cells are thought to be precursor mesodermal cells because similar cells are found to reside at dissepiments. This assumption is based exclusively on histological sections analysis. Furthermore, cells of this type have been reported only in a few clitellate annelids, particularly oligochaetes (Boilly, 1969b; Hill, 1970; Paulus and Muller, 2006). Randolph who described such cells in *Lumbriculus* and called them neoblasts (Randolph, 1891, 1892), suggested, in modern terms, a stem cell function for these cells. However, the use of this term was abandoned in the context of annelids, and only for

several recent years the attempts to bring attention back to this term have been made, arguing for the existence of neoblast-like stem cells in annelids. At present this term refers to a unique line of pluripotent cells in planarians which is the only source for maintenance for both somatic and germ cell lineages in these animals. On the one hand, specific morphological features of annelid neoblasts make weak specialization of these cells evident; on the other hand, these cells are species-specific and not common across annelid groups, which is confusing for the investigator.

Interestingly, in asexual reproduction cell migration does occur, although to a less extent. For example, according to our data, migration of branched cells precedes the fission zone development in the oligochaete worm. As these cells do not express the markers of the stem or pluripotent cell, their functions are probably not related to blastema formation but may be connected to signal transduction, including cues for positional information (Babakhanova and Kostyuchenko, unpublished).

It is well known that wound healing is critically important for the progress of regeneration, when a temporary barrier to the external environment is established providing conditions necessary for regeneration (Stocum, 2012). In most annelids, the cut edges of the epidermal epithelium fuse to each other forming the wound epithelium (Herlant-Meewis, 1964; Korotkova, 1997). The edges of gut epithelium also fuse each other forming blindly closed intestine. However, this is not the case for all species across the phylum. For example, in the polychaetes *Sabella* (Hill, 1970) and *Nereis* (Boilly, 1969a) edges of the epidermis fuse directly to the edges of the gut epithelium. In this case a wound epithelium is not formed and gut stays open during the regeneration. Re-epithelization is completed within the first day, usually prior the epidermal cell start to proliferate actively, thus, occurring by rearrangement of cells (Hill, 1970; Kharin et al., 2006; Paulus and Muller, 2006; Zattara and Bely, 2011).

Although it is a rare exception, regeneration in annelids can manage without apparent formation of the wound epithelium but yet epithelization through wound healing takes place. According to our data, in rare cases when wounds do not heal or the served edges of the gut and epidermal epithelium do not fuse each to other, the animal dies without regenerative bud formation. This argues for epithelization as being the earliest and critically important morphogenetic event during restoration of the lost structures. Such is not the case for asexual reproduction (table). Even during architomy, when new organs regenerate after the splitting of the body, no wound epithelium is observed (Martinez et al., 2005). In paratomy, organ differentiation precedes fission termination, allowing the physical separation of the two daughter zooids to complete without a wound (Ivanova-Kazas, 1977; Kharin et al., 2006; Babakhanova et al., 2012). It is worth noting,

**Table 1.** Comparison of the main morphogenetic events during the head formation between regeneration and the two types of transverse fission, architomy and paratomy

Event	Anterior part of the body formation during		
	regeneration	architomy	paratomy
Splitting of the body	Occurs prior to regeneration	Occurs after the beginning of architomy	Terminates the process
Wound healing	Starts soon after amputation, lasts for approximately 24 h	Poorly apparent, if any occurs after splitting	Not observed even when paratomy is complete
Cell migration towards the wound site or fission zone	Starts after amputation, before wound epithelium formation	Probably starts prior to splitting	Precedes fission zone formation
Modification of epidermal cells	Limited, occurs during wound healing	Limited, occurs prior to splitting and during wound healing	Apparent, involves almost all epidermal layer of the fission zone, starts at the onset of the process, persists till its termination
Active proliferation of epidermal cells	Starts at the end of wound healing stage and persists through the growth of a regenerate	Starts before splitting and persists through the regeneration of missing structures	Starts at the onset of epidermal cell modification, persists through the process, decreasing by its termination
Blastema formation	Starts next to the onset of active proliferation of epidermal cells; blastema expands noticeably by the beginning of segmentation	Blastema formation precedes splitting, further growth may occur until segmentation starts	Starts after the onset of active epidermal cells proliferation, blastema expands noticeably by the beginning of segmentation
Segmentation and segment positional identity	Oftenly hypomorphic, although the formation of species-specific number of anterior segments is usually accomplished by morphallaxis of old segments adjacent to the wound	Oftenly hypomorphic, old segments undergo morphallactic reorganisation with regard to their new position along the anterior-posterior (AP) axis	The species-specific number of anterior segments is formed by blastema cells; limited morphallaxis of old segments, if present, precedes fission zone formation and persists through the process
Muscular system	Muscles are ruptured; as the blastema grows, terminal ends of longitudinal fibers elongate while circular muscle fibers are formed <i>de novo</i>	Muscles undergo some changes to facilitate splitting of a worm into fragments	Muscles undergo some changes to facilitate splitting of new zooids

**Table 1.** (Contd.)

Event	Anterior part of the body formation during		
	regeneration	architomy	paratomy
Digestive system	Transient reorganisation of the digestive system includes loss of cilia near the wound site and stomach formation (if any) through morphallaxis	Morphallactic digestive system reorganisation along the AP axis	Growth and elongation of the gut at the fission zone with differentiated state persisted; the gut lumen shrinks at fission plane and a new stomach emerges by morphallaxis in the posterior zooid at late stages of fission
Nervous system	Following ventral nerve cord (VNC) rupture, the severed VNC elongates, new nerve fibers grow out, cerebral commissures and VNC ganglia form	VNC changes, including those at the molecular level precede splitting; after splitting new nerves, cerebral commissures and VNC ganglia develop	VNC changes precede splitting; the changes include transient peripheral nerves formation, new ganglia and cerebral commissures development; VNC is only ruptured when splitting occurs
Changes at the molecular level	Start immediately after amputation	Precede splitting (from several days to 1 week)	Precede morphologically observable fission zone formation and persist through its growth and differentiation

After Berrill, 1952; Christensen, 1959; Herlant-Meewis, 1964; Kasinov, 1962, 1970; Ivanova-Kazas, 1977; Korotkova, 1997; Myohara et al., 1999; Bely, 1999, 2006; Martinez et al., 2005; Kharin et al., 2006; Smirnova and Kostyuchenko, 2007; Bely and Sikes, 2010; Steinmetz et al., 2010; Zattara and Bely, 2011, 2013; Babakhanova et al., 2012; Kostyuchenko, 2012; Kostyuchenko et al., 2012; Myohara, 2012; Sugio et al., 2012; Kozin and Kostyuchenko, 2015.

however, that epithelial folds, which separate adjacent zooids, are derived from local epidermal epithelial cells undergoing proliferation at the fission zone (Kharin et al., 2006; Babakhanova et al., 2012; Kostyuchenko, 2012; Kostyuchenko et al., 2012). Thus, wound healing and epidermal morphogenesis observed in the paratomic fission zone may have much more in common than we have presumed.

It is known that following an amputation the extensive changes in the cell interactions with the extracellular matrix as well as in the intercellular contacts are observed at the site of injury (Stocum, 2012). Muscle tissue undergo essential alteration including degeneration. Cells of both superficial and internal tissues begin to proliferate. All these lead to rearrangement and migration of cells (required for regenerative blastema formation or morphallaxis initiation) (Fontes et al., 1983; Coulon et al., 1989; Dupin et al., 1991; Korotkova, 1997). Detailed morphological descriptions of the early and middle stages of the fission also suggest active cell migration and proliferation at the fission zone. It is known that all three germinal layers can contribute to the development of blastema mass (Kharin et al., 2006; Babakhanova et al., 2012; Kostyuchenko et al., 2012). The enhanced activity of

epidermal cells is of particular interest because the cells take on since of undifferentiated state and actively divide, with the progeny probably migrating inside the body (Kharin et al., 2006; Smirnova and Kostyuchenko, 2007; Babakhanova et al., 2012; Kostyuchenko, 2012; Kostyuchenko et al., 2012). Altogether, these events may eventually drive local changes in the extracellular matrix and intercellular contacts.

Interaction of tissues at the wound site or at the fission zone appear to be critical for initiating of blastema formation. A number of studies reported that the injured end of the ventral nerve cord can activate regeneration and proliferation (Avel, 1961; Coulon and Thouveny, 1984; Muller et al., 2003). Herlant-Meewis and Deligne (1965) proposed the trophic/neuroregulatory role of neurotransmitters in wound healing and blastema cell division in *Lumbricidae*. Oligochaete *Enchytraeus japonensis*, which reproduces by architomy, was shown to grow nerve fibers innervating the whole blastema prior to the onset of regeneration process (Yochida-Noro et al., 2000). Changes in the nerve system during the formation of new zooids are evidence for progressive development and suggest the possible role of the nerve system in asexual reproduc-

tion (Martinez et al., 2005; Kharin et al., 2006; Zattara and Bely, 2011).

It cannot be excluded, however, that other body systems influence patterning and formation of anlagen of organs and body parts. As was recently reported, the expression of numerous genes providing positional information control during planarian regeneration, is found in muscle cells but not in neoblast. This suggest the specific role of the musculature for re-patterning of the planarian body (Witchley et al., 2013). In annelid studies, considerable data were obtained arguing for a key role of the gut and epidermal epithelia (Korotkova, 1997). However, the issue is that the general conclusions cannot be made up based on scattered descriptions. Even studies on closely related species, probably due to differences in experimental setups, report conflicting findings with regard to the role of certain old tissues in initiating blastema formation.

### BLASTEMA IN REGENERATION AND ASEXUAL REPRODUCTION

Both blastema formation and morphallactic reorganization of the body during regeneration and asexual reproduction obviously occur under control of the pre-existing tissues which also act as a cell source for at least early stages of development. Most investigators agree that all three germ layers contribute to the regenerative blastema. In addition, it is strongly suggested that at least a few oligochaete species also share this feature as applied to paratomic fission. This conclusion is supported by results of experiment on thymidine-analog incorporation and mitotic markers labeling (Boilly, 1969a; Hill, 1970; Smirnova and Kostyuchenko, 2007; Zattara and Bely, 2011, 2013; Babakhanova et al., 2012). Thus, DNA synthesis was detected by *BrdU* assay in various tissues in *Pristina longiseta* during both regeneration and asexual reproduction, that suggests is high proliferative potency of the cells (Smirnova and Kostyuchenko, 2007; Babakhanova et al., 2012; Kostyuchenko, 2012; Kostyuchenko et al., 2012).

However, it is still unclear whether dedifferentiation of pre-existing tissues or probable existence of stem cell population plays crucial role in asexual reproduction and regeneration. Whereas some authors, for instance, Sapaev (1978), advocate for large-scale dedifferentiation of adult tissues during the morphogeneses and argue against the presence of somatic stem cell population, others suggest the existence of primarily undifferentiated cells called “neoblasts” as a source of organ formation, analogously to planarian proliferative somatic cell population (Zhinkin, 1934; O’Brien, 1942; Kasinov, 1963; Bilello and Potswald, 1974; Sugio et al., 2012). Ultrastructural examination of the fission zone in *Chaetogaster lymnaei*, conducted by Sapaev (1976, 1978) has revealed that subesophageal ganglion is formed *de novo* from dedifferentiated cells of the ventral epidermis. On the contrary, our data give evidence that in *Nais communis* and *Pristina longiseta*

development of new subesophageal ganglion is based on both dedifferentiated cells and pre-existing elements of the nervous system (Kharin et al., 2006). Sapaev, however, reported that gut epithelial cells undergo dedifferentiation in *Ch. lymnaei*, while the gut epithelium in *Nais* and *Pristina* remains differentiated. Nevertheless, we observed an accumulation of undifferentiated cells in the fission zone in *N. communis* and *P. longiseta*, with differentiated cells being hardly detectable.

Our data suggest migration of dedifferentiated epidermis progeny into the segment followed by numerous cell divisions which then result in blastema formation (Kharin et al., 2006; Smirnova and Kostyuchenko, 2007; Babakhanova et al., 2012; Kostyuchenko, 2012; Kostyuchenko et al., 2012). Epidermal cells at fission zone were observed not only to have ultrastructural characteristics but also expression patterns of pluripotency markers *vasa*, *pl10*, and *piwi* very similar to that of blastema cells. Upregulation of these gene expression likewise occurs in the wound epithelium, inner cells of the newly regenerated parts, and the neighboring old epithelium (Smirnova and Kostyuchenko, 2007; Kostyuchenko, 2012; Kostyuchenko et al., 2012; Kozin and Kostyuchenko, 2015). Therefore, blastema in asexual reproduction of oligochaetes is likely to originate partially from the dedifferentiated epidermal cells. However, according to available data on regeneration the opportunity that other cells which may also be of stem cell population participate in the process cannot be excluded. To address this issue, a complex approach is required, with tracing of cell lineages, revealing of cell population dynamics, inhibition analysis and identification of pluripotency and differentiated state of cells performed.

To answer the question about cell sources of blastema, investigation on broad range of annelid species has to be carried out. Although, it is evident that even closely related species may differ drastically in asexual reproduction and regeneration features. For example, neoblast-like cells are required for regeneration of *Enchytraeus japonensis* (Sugio et al., 2012), whereas *Enchytraeus buchholzi* does not bear such cells at all (Myohara, 2012). Thus, variability across even one genus in both morphology and genetic regulatory networks can be expected.

### UNIVERSAL MECHANISMS: REGIONAL SPECIFICATION ALONG THE ANTERIOR–POSTERIOR AXIS

In our discussion of interactions between tissues during regeneration, we have noted the key role of signal molecules and signal centres for the correct axial polarity establishment and also for setting up the specific molecular state of cells composing the regenerate or fission zone, maintained by expression of genes encoding stem- and multipotent-cell markers. Although regeneration, architomy, and paratomy occur mostly by epimor-

phosis accompanied with active proliferation, morpholactic transformation of the old tissues and even whole segments are observed during a new head region formation (table). The re-building of the old segments is in accordance with their new position in the body. This is evidence of the existence of some positional signals disturbance and normalization which play a key role for both morphogenesis and activation of the regenerative processes.

However, it is not possible to find the general pattern determining the segment competence for anterior regeneration and fission zone formation by using morphological data only. In oligochaetes and polychaetes, ability for anterior regeneration is characteristic not for the most species and is also limited mainly to anterior part of body. Transverse amputation level shift along the anterior–posterior axis may significantly affect the course and outcome of anterior regeneration and even perturb the head-versus-tail regeneration choice (Korotkova, 1997). The head regenerative territories are species-specific. The published data do not allow making general conclusions for the phylum. Asexual reproduction is another issue that calls for further research efforts. During paratomy, a fission zone develops in the certain segment in some species, whereas during stolonization a particular proliferative segment is formed. Other species, for example *N. communis* and *P. longisetata*, lack a specialized segment but possess a competent region within which fission is predominantly triggered (Christensen, 1959; Kasinov, 1962, 1970; Ivanova-Kazas, 1977; Myohara et al., 1999; Kharin et al., 2006). Mathematical modeling of agametic reproduction in oligochaetes reveals that both the specific segment for paratomic fission (Kasinov, 1962, 1970) and the competent region (Kharin et al., 2006) are established by complex positional identity signal system within the body. Hence, the fission zone position is somehow predetermined and may be controlling mechanisms are bound with gradients of physiological and/or morphogenetic factors.

Recently, a progress has been made in understanding the molecular components making the putative gradients. Extensive changes occur not only at the anatomical and physiological levels, but also at the molecular levels without apparent morphological changes. Thus, during initiating of architomy in *L. variegatus*, some neuronal-specific markers are expressed several days prior to separation of fragments. In particular, the anterior–posterior gradient-like patterns of mannose-rich neutral glycoproteins of different molecular weight overlapped in prospective fission zone were detected in animals before splitting. In animals stimulated to asexual reproduction, a local change of this proteins distribution occurs, with head-specific proteins allocated at the presumptive head region (Martinez et al., 2005).

Undoubtedly, *Hox* genes provide putative signals required for positional identity in regeneration and asexual reproduction. Novikova et al. (2013) reported

expression patterns of these genes during the posterior regeneration in the polychaete worm *Alitta virens*. *Hox* gene activity lacks temporal colinearity in newly formed segments of *A. virens*, expression of various genes being different. *Lox5*, *Lox2*, and *Post2*, which patterns are typical of the posterior body end, are upregulated in segments that became the most posterior due to tail amputation, as early as at 4 hours after the treatment. It is likely that new posterior boundaries of the body are established by this. These genes, like some others, change their expression levels not only in ectoderm but, predominantly, in neural system. *Hox2* and *Hox3* are expressed in the growth zone during normal growth, and the first sign of their activity at the wound site become detectable later. However, 24 hours post amputation, when according to our data the high cell proliferation starts, activity of these genes is dramatically upregulated and is fully restored by the time of new terminal structures formation. The late response genes, *Hox1* and *Hox4*, do not participate in re-patterning of the body boundaries at early stage, but are reactivated in response to an injury after the initiation of blastema formation and the gradual restoration of lost structures. Based on these findings, Novikova et al. (2013) conclude that the regeneration processes in *A. virens* are comprised of two phases. During the first phase, the positional information and the whole positional identity are reorganized due to changes in *Hox* gene expression and then restored within the new body boundaries. This reorganization begins prior to blastema formation and is observed in differentiated cells of old segments. During the second phase, all *Hox* genes are expressed and take part in patterning of terminal structures and new segments (Novikova et al., 2013). Thus, these genes may be involved in the establishment, maintenance, and correct restoration of the positional memory in the worm.

Our results of cloning and expression pattern analysis of regional specification genes in *P. longisetata* and *N. communis* revealed putative duplications of *Hox* gene homologues in their genomes. Although they demonstrate spatial colinearity of expression, the expected pattern is complicated with the presence of homologues and splice-variants which are expressed at different time windows (Kostyuchenko, 2012; Kostyuchenko, unpublished). The expression pattern is disrupted at the initiating of fission, the first signs detectable long before blastema growth becomes apparent. Once blastema formation is complete, the expression of *Hox* genes re-establishes the usual pattern within each daughter zooid. Notably, anterior boundaries of *Hox* genes expression in the anterior zooid and posterior boundaries in the posterior one remain unaltered. Levels of expression of these genes in the paratomic fission zone are similar to those in polychaete larval body (Steinmetz et al., 2011). According to in situ hybridization results, disruption of *Hox* gene expression in the fissioning segment occurs immediately after the activation of the two

homeobox genes, *six3* and *otx1* in the same region. *Plo-six3* and *Plo-otx1* expression becomes detectable at the onset of fission zone formation and anterior regeneration and persists until the accomplishment of the processes in *P. longiseta*. The expression domains are primarily located in epidermis. Later on, while *Plo-six3* expression continues in the epidermis of the anterior region of developing head, it is also detected in two deep cells of the anterior part of developing brain. At the same time, *Plo-otx1* is mainly expressed in the inner cell mass (Steinmetz et al., 2010). Thus, these two genes, along with *Hox* genes, constitute positional information for different regions of the body of daughter zooids. Interestingly, they also demonstrate striking similarities in body patterning to those during embryonic, larval, and post-embryonic development of annelids, suggesting universal mechanisms of positional specification and indirectly supporting the theory of heteronomous segments in annelids (Iwanoff, 1928).

#### SEARCHING FOR SHARED MECHANISMS

Species capable of regeneration have been reported for most annelid subgroups, with enhanced regeneration abilities in asexually reproducing animals. However, regeneration abilities can vary widely from whole body regeneration to complete loss of this ability even in closely related species. It seems to be likely that changes of some subsets in the genetic regulatory network can lead to both the decrease and increase in regenerative abilities. Such changes are often conserved through evolution. Therefore, mechanisms of regeneration could be understood with a comparative analysis of multiple parameters of regeneration and similar processes such as, first of all, asexual reproduction. Meanwhile, there is no model annelid to study regeneration because of the lack of integrated studies conducted on any given annelid species. This is mainly due to the fact that relevant studies differ in design of experiments, species studied, and techniques used (being often out-of-date and inaccurate). Studies of the cellular and molecular mechanisms are only emerging now. It is no doubt that new integrated approaches to exploring regeneration and asexual reproduction with the use of modern methods will provide new insights into cell fate plasticity, initiating mechanisms of regeneration, and evolution of post-embryonic development. It is, thereby, important to further investigate molecular and functional aspects of genetic regulatory networks guiding regeneration and asexual reproduction and, in particular, conserved network components called kernels.

#### ACKNOWLEDGMENTS

This study was supported by grant no. 1.38.209.2014 from St. Petersburg State University. We also acknowledge the Research Resource Center for Molecular and

Cell Technologies, St. Petersburg State University, for technical support.

#### REFERENCES

- Avel, M., L'influence du système nerveux sur la régénération chez les urodeles et les oligochètes, *Bull. Soc. Zool. Fr.*, 1961, vol. 86, p. 464.
- Babakhanova, R.A., Smirnova, N.P., and Kostyuchenko, R.P., Dedifferentiation of somatic cells as a probable source of recovery of missing structures in asexual reproduction in annelids, *Tsitologiya*, 2012, vol. 54, no. 4, pp. 334–335.
- Bely, A., Decoupling of fission and regenerative capabilities in an asexual oligochaete, *Hydrobiologia*, 1999, vol. 406, pp. 243–251.
- Bely, A. and Wray, G., Evolution of regeneration and fission in annelids: insights from engrailed and orthodenticle-class gene expression, *Development*, 2001, vol. 128, pp. 2781–2791.
- Bely, A.E., Distribution of segment regeneration ability in the Annelida, *Integr. Comp. Biol.*, 2006, vol. 46, pp. 508–518.
- Bely, A.E. and Sikes, J.M., Latent regeneration abilities persist following recent evolutionary loss in asexual annelids, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, vol. 107, pp. 1464–1469.
- Berrill, N.J., Regeneration and budding in worms, *Biol. Rev.*, 1952, vol. 27, pp. 401–438.
- Bilello, A. and Postwald, H., A cytological and quantitative study of neoblasts in the naid *Ophidonais serpentina* (Oligochaeta), *Wilhelm Roux' Arch. Dev. Biol.*, 1974, vol. 174, pp. 234–249.
- Boilly, B., Origine des cellules régénératrices chez *Nereis diversicolor* O.F. Muller (Annelide Polychete), *Wilhelm Roux's Arch. Dev. Biol.*, 1969a, vol. 162, pp. 286–305.
- Boilly, B., Sur l'origine des cellules régénératrices chez les annélides polychètes, *Arch. Zool. Exp. Gen.*, 1969b, vol. 110, pp. 127–143.
- O'Brien, J.P., Studies on effects of x-rays on regeneration in the fragmenting oligochaete *Nais paraguayensis*, *Growth*, 1942, vol. 6, pp. 203–229.
- Christensen, B., Asexual reproduction in the *Enchytraeidae* (Oligochaeta), *Nature*, 1959, vol. 184, no. 4643, pp. 1159–1166.
- Cornec, J.P., Cresp, J., Delye, P., Hoarau, F., and Reynaud, G., Tissue responses and organogenesis during regeneration in the oligochaete *Limnodrilus hoffmeisteri* (Clap.), *Can. J. Zool.*, 1987, vol. 65, pp. 403–414.
- Coulon, J. and Thouveny, Y., Relation entre l'innervation et l'activité prolifératrice des cellules blastématique au cours de la régénération de l'annelide polychete *Owenia fusiformis*: études ultrastructurale et autoradiographique, *Arch. Anat. Microsc.*, 1984, vol. 73, pp. 45–56.
- Coulon, J., Diano, M., Arsanto, J.-P., and Thouveny, Y., Remodeling processes during anterior regeneration of *Owenia fusiformis* (Polychaeta, Annelidae): a morphological and immunocytochemical survey, *Can. J. Zool.*, 1989, vol. 67, pp. 994–1005.
- Dupin, F., Coulon, J., Le Parco, Y., Fontes, M., and Thouveny, Y., Formation of the extracellular matrix during the epimorphic anterior regeneration of *Owenia fusiformis*:



- autoradiographical and in situ hybridization studies, *Int. J. Dev. Biol.*, 1991, vol. 35, pp. 109–119.
- Fontes, M., Coulon, J., Delgrossi, M.H., and Thouveny, Y., Muscle dedifferentiation and contractile protein synthesis during post-traumatic regeneration by *Owenia fusiformis* (polychaete annelid), *Cell. Differ.*, 1983, vol. 13, pp. 267–282.
- Gibson, G.D. and Paterson, I.G., Morphogenesis during sexual and asexual reproduction in *Amphipolydora vestalis* (Polychaeta: Spionidae), *New Zealand J. Mar. Fres. Res.*, 2003, vol. 37, pp. 741–752.
- Herlant-Meewis, H., Regeneration in annelids, *Adv. Morphogen.*, 1964, vol. 4, pp. 155–215.
- Herlant-Meewis, H. and Deligne, J., Influence of the nervous system on regeneration in annelids, in *Regeneration in Animals and Related Problems*, Amsterdam: North-Holl. Publ. Co., 1965, pp. 228–239.
- Hill, S.D., Origin of the regeneration blastema in polychaete annelids, *Am. Zool.*, 1970, vol. 10, pp. 101–112.
- Ivanova-Kazas, O.M., *Bespoloe razmnozhenie zhivotnykh* (Asexual Reproduction of Animals), Leningrad: Izd. LGU, 1977.
- Iwanoff, P.P., Die entwicklung der Larvalsegmente bei den Annelide, *Z. Morph. Oekol. Tiere*, 1928, Bd. 10, pp. 62–161.
- Kasinov, V.B., Linear increments of zooids in asexual reproduction of some oligochaetes, *Dokl. Akad. Nauk SSSR*, 1962, vol. 143, no. 4, pp. 967–980.
- Kasinov, V.B., Neoblasts and development of the posterior regenerate in lower oligochaetes, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Leningrad: Len. Gos. Univ. im. A.A. Zhdanova, 1963.
- Kasinov, V.B., Autoregulation of asexual reproduction in oligochaetes, *Ontogenez*, 1970, vol. 1, pp. 147–156.
- Kawamoto, S., Yoshida-Noro, C., and Tochinai, S., Bipolar head regeneration induced by artificial amputation in *Enchytraeus japonensis* (Annelida, Oligochaeta), *J. Exp. Zool. Pt A Comp. Exp. Biol.*, 2005, vol. 303A, pp. 615–627.
- Kharin, A.V., Zagainova, I.V., and Kostyuchenko, R.P., Formation of the paratomic fission zone in freshwater oligochaetes, *Russ. J. Dev. Biol.*, 2006, vol. 37, no. 6, pp. 354–365.
- Korotkova, G.P., *Regeneratsiya zhivotnykh* (Regeneration of Animals), St. Petersburg: Izd. SPbGU, 1997.
- Kostyuchenko, R.P., Kozin, V.V., Smirnova, N.P., and Babakhanova, R.A., Formation of fission zone and growth zone as multipotent cell populations in development and regeneration of annelids, in *Euro Evo Devo Abstract Book*, 2012, p. 193.
- Kostyuchenko, R.P., Molecular and cellular events of AP axis and tissue transformations during asexual reproduction in naidid oligochaetes, *Euro Evo Devo Lisbon Abstract Book*, 2012, p. 87.
- Kozin, V.V. and Kostyuchenko, R.P., *Vasa*, *PL10*, and *Piwi* gene expression during caudal regeneration of the polychaete annelid *Alitta virens*, *Dev. Genes Evol.*, 2015, vol. 225, pp. 129–138.
- Lesiuk, N. and Drewes, C., Autotomy reflex in a freshwater oligochaete, *Lumbriculus variegates*, *Hydrobiologia*, 1999, vol. 406, pp. 253–261.
- Liu, S.-Y., Selck, C., Friedrich, B., Lutz, R., Vila-Farré, M., Dahl, A., Brandl, H., Lakshmanaperumal, N., Henry, I., and Rink, J.C., Reactivating head regrowth in a regeneration-deficient planarian species, *Nature*, 2013, vol. 500, pp. 81–84.
- Martinez, V.G., Menger, G.J., 3rd, and Zoran, M.J., Regeneration and asexual reproduction share common molecular changes: upregulation of a neural glycoepitope during morphallaxis in *Lumbriculus*, *Mech. Dev.*, 2005, vol. 122, no. 5, pp. 721–732.
- Morgan, T.H., *Regeneration*, Columbia Univ. Biol. Ser. Norwood, MA: Macmillan, 1901.
- Muller, M.C.M., Berenzen, A., and Westheide, W., Experiments on anterior regeneration in *Eurythoe complanata* (“Polychaeta,” Amphinomididae): reconfiguration of the nervous system and its function for regeneration, *Zoomorphology*, 2003, vol. 122, pp. 95–103.
- Myohara, M., Yochida-Noro, C., Kobari, F., and Tochinai, S., Fragmenting oligochaete *Enchytraeus japonensis*: a new material for regeneration study, *Dev. Growth Differ.*, 1999, vol. 41, no. 5, pp. 549–555.
- Myohara, M., What role do annelid neoblasts play? A comparison of the regeneration patterns in a neoblast-bearing and a neoblast-lacking enchytraeid oligochaete, *PLoS One*, 2012, vol. 7, no. 5, p. e37319.
- Novikova, E.L., Bakalenko, N.I., Nesterenko, A.Y., and Kulakova, M.A., Expression of *Hox* genes during regeneration of nereid polychaete *Alitta (Nereis) virens* (Annelida, Lophotrochozoa), *EvoDevo*, 2013, vol. 4, no. 14.
- Paulus, T. and Muller, M.C.M., Cell proliferation dynamics and morphological differentiation during regeneration in *Dorvillea bermudensis* (Polychaeta, Dorvilleidae), *J. Morphol.*, 2006, vol. 267, pp. 393–403.
- Randolph, H., The regeneration of the tail in *Lumbriculus*, *Zool. Anz.*, 1891, vol. 14, pp. 154–156.
- Randolph, H., The regeneration of the tail in *Lumbriculus*, *J. Morphol.*, 1892, vol. 7, pp. 317–344.
- Sánchez Alvarado, A., Regeneration in the metazoans: why does it happen?, *BioEssays*, 2000, vol. 22, pp. 578–590.
- Sapaev, E.A., Essays on the morphology and biology of two subspecies *Shaetogaster lymnaei*, Baer, 1827, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Kazan: KGU, 1976.
- Sapaev, E.A., Ultrastructural aspects of morphogenetic processes in the posterior part of the paratomy zone of the oligochaete *Chaetogaster lymnaei*, *Tsitologiya*, 1978, vol. 20, no. 2, pp. 149–153.
- Sikes, J.M. and Newmark, P.A., Restoration of anterior regeneration in a planarian with limited regenerative ability, *Nature*, 2013, vol. 500, pp. 77–80.
- Smirnova, N.P. and Kostyuchenko, R.P., Cellular sources of paratomy zone in oligochaetes *Pristina longiseta* (Naididae): cloning and analysis of expression of genes markers of stem and undifferentiated cells, *Tsitologiya*, 2007, vol. 49, no. 9, p. 794.
- Steinmetz, P.R.H., Urbach, R., Posnien, N., Eriksson, J., Kostyuchenko, R.P., Brena, C., Guy, K., Akam, M., Bucher, G., and Arendt, D., *Six3* demarcates the anterior-most developing brain region in bilaterian animals, *EvoDevo*, 2010, vol. 1, no. 14, pp. 1–9.
- Steinmetz, P.R.H., Kostyuchenko, R.P., Fischer, A., and Arendt, D., The segmental pattern of *otx*, *gbx*, and *Hox*

- genes in the annelid *Platynereis dumerilii*, *Evol. Dev.*, 2011, vol. 13, no. 1, pp. 72–79.
- Stocum, D.L., *Regenerative Biology and Medicine*, 2nd ed., Amsterdam: Elsevier Sci. Publ. Co. Inc., 2012.
- Sugio, M., Yoshida-Noro, C., Ozawa, K., and Tochinai, S., Stem cells in asexual reproduction of *Enchytraeus japonensis* (Oligochaeta, Annelid): proliferation and migration of neoblasts, *Dev. Growth Differ.*, 2012, vol. 54, no. 4, pp. 439–450.
- Umesono, Y., Tasaki, J., Nishimura, Y., Hrouda, M., Kawaguchi, E., Yazawa, S., Nishimura, O., Hosoda, K., Inoue, T., and Agata, K., The molecular logic for planarian regeneration along the anterior-posterior axis, *Nature*, 2013, vol. 500, pp. 73–76.
- Vorontsova, M.A. and Liosner, L.D., *Asexual Propagation and Regeneration*, London: Pergamon Press, 1960.
- Witchley, J.N., Mayer, M., Wagner, D.E., Owen, J.H., and Reddien, P.W., Muscle cells provide instructions for planarian regeneration, *Cell Rep.*, 2013, vol. 4, pp. 633–641.
- Yochida-Noro, C., Myohara, M., Kobari, F., and Tochinai, S., Nervous system dynamics during fragmentation and regeneration in *Enchytraeus japonensis* (Oligochaeta, Annelida), *Dev. Genes Evol.*, 2000, vol. 210, pp. 311–319.
- Zattara, E.E. and Bely, A.E., Evolution of a novel developmental trajectory: fission is distinct from regeneration in the annelid *Pristina leidyi*, *Evol. Dev.*, 2011, vol. 13, pp. 80–95.
- Zattara, E.E. and Bely, A.E., Investment choices in post-embryonic development: quantifying interactions among growth, regeneration, and asexual reproduction in the annelid *Pristina leidyi*, *J. Exp. Zool. Pt B Mol. Dev. Evol.*, 2013, vol. 320, pp. 471–488.
- Zhinkin, L., Über die Bedeutung der Mesodermanlagen bei der Regeneration von *Rhynchelminis limosella* (Untersuchungen über die Wirkung der Röntgenstrahlen auf regeneration und Transplantation), *Zool. Anz.*, 1934, Bd 105, pp. 305–312.

*Translated by A. Sprygin*