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Review

Biology of regeneration in earthworms: mechanisms, factors and molecular insights. A review

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Abstract
Earthworms are ecosystem engineers that play a vital role in soil structure, nutrient cycling, and ecological balance. Beyond their ecological importance, they exhibit remarkable regenerative potential, making them valuable models for studying cellular and molecular reprogramming. Regeneration in earthworms follows a coordinated sequence of wound healing, blastema formation, cellular proliferation, re-segmentation, and tissue growth. These processes are regulated by conserved gene networks and signalling pathways, including pluripotency-associated factors such as Sox2, Oct4, Nanog, Lin28, and c-Myc, along with developmental regulators like Hox and Wnt families. The clitellum serves as a reservoir of stem cells and regenerative factors (e.g., TCTP, Wnt3a, VEGF, YAP1), coordinating cell proliferation and morphogenesis. Species-specific differences exist: Eisenia fetida and Eudrilus eugeniae can regenerate both anterior and posterior segments, whereas Lumbricus terrestris shows limited capacity. Regeneration is influenced by intrinsic and extrinsic factors, with pollutants such as pesticides and heavy metals impairing gene expression and promoting oxidative stress. Understanding these molecular and ecological determinants highlights the dual significance of earthworms as models for regenerative biology and as indicators in ecotoxicology. This review synthesizes current knowledge and emphasizes future prospects for applying earthworm regeneration studies to biomedical and ecological contexts.

Highlights:

- *Earthworms exhibit complex and intricately coordinated multi-stage regenerative mechanisms*
- *Clitellum serves as a biological reservoir of stem cells and molecular signalling factors that govern tissue renewal*
- *Pluripotency genes (Sox2, Oct4, Nanog) modulates blastema formation*
- *Environmental pollutants significantly impair regenerative processes*
- *Earthworms serve as a model organism for studies in regenerative medicine and ecotoxicology*

Keywords: *annelid biology, blastema formation, clitellum, earthworm regeneration, environmental stress, pesticide toxicity, Sox2, Oct4, stem cell reprogramming*

List of Abbreviations:
BMP- Bone Morphogenetic Protein
c-Myc- Cellular Myelocytomatosis Viral Oncogene
DEG(s)- Differentially Expressed Gene(s)
EGFR- Epidermal Growth Factor Receptor
ES- Embryonic Stem (cells)
EST- Expressed Sequence Tags
FGF- Fibroblast Growth Factor
LINE2- Long Interspersed Nuclear Element 2
Lin28- MicroRNA-binding protein coding gene
Nanog- Homeobox transcription factor Nanog

NGF- Nerve Growth Factor
Oct4- Octamer-binding transcription factor 4
Pax- Paired box gene family
Pex-lab01, Pex-lab02, Pex-lab03- Labial Hox genes in *Eisenia fetida*
RCF- Regenerative Clitellum Factors
Sox2- Sex-determining region Y-box 2 transcription factor
TCTP- Translationally Controlled Tumor Protein
VEGF- Vascular Endothelial Growth Factor
VNC- Ventral Nerve Cord
Wnt- Wingless/Integrated signalling family
YAP1- Yes-Associated Protein 1

INTRODUCTION

Earthworms, belonging to the class Oligochaeta of the Phylum Annelida, are one of the most significant soil organisms, playing key roles in soil ecosystem dynamics, and maintenance of soil quality and fertility parameters. Earthworms are distributed worldwide, and their population is considered to make up approximately 8% of the total biomass of the soil organisms [1]. The ecological significance of earthworms has been known for hundreds of years, and several scientific results have established the earthworms as soil ecosystem engineers [2,3]. As important soil organisms, earthworms support nutrient cycling, enhance soil structure, and stimulate microbial activity [4]. Their burrows provide aeration of the soil, allowing the growth of the roots, and the infiltration of water into the soil helping the plants to produce even better. They help break down organic materials as decomposers, which improves the soil with nourishing nutrients [4]. Earthworms play two major roles in the soil ecosystem, namely soil formation and nutrient cycling [5,6], which are two vital components of soil ecosystem dynamics. These soil animals are also involved in ecological interactions with other biotic and abiotic factors required for the sustenance of soil ecosystem functioning, along with other important contributions in the remediation and restoration of ecosystem balance [4]. Earthworms are soil-dwelling animals; hence, they are very much prone to serious damage and wounds inflicted on them, and therefore, they have a great regenerative capacity, which helps in their survival and maintenance of their population structure and function [7].

There are three major ecological or functional groups of earthworms: epigeic, anecic and Endogeic, based on their morphology, and their foraging behaviour, endogeic earthworms live and feed in the soil, epigeic earthworms mainly live and feed on the leaf litter at the soil surface, anecic earthworms make vertical burrows in soil and feed on leaf litter which they drag into their burrows [8, 9]. Earthworms are of primary importance for ecosystem functioning because they modify the availability of resources for other organisms through physical and chemical changes in their surrounding soil environment [10, 11]. As a consequence, they carry out numerous soil-based ecosystem services [4].

The earthworm is a unique and valuable model for investigating the mechanism of regeneration [12]. Regeneration is the ability of the organism to restore or regain lost body parts [13]. In the animal kingdom, regeneration capability varies from species to species, and it ranges from the ability to regenerate the whole body to a limited ability to restore only a particular organ [14]. Hydra, annelids, molluscs, nemertean worms, planarians, chordates, and vertebrates are some of the well-known examples with diverse regeneration capability [15]. Basically, like other invertebrates, annelids possess the regeneration ability, and among them, earthworms are able to regenerate whole body segments [16, 17]. In earthworms, 2 distinct regeneration phenomena are observed: one group of species depend on the clitellum for their regeneration, and the other group of worms does not need the clitellum for their regeneration [18].

The regeneration procedure in earthworms basically involve five stages: Wound healing, Blastema formation, Blastema differentiation, Resegmentation and Growth. To initiate regeneration, wound healing is the very initial step, which helps to prevent further tissue damage as well as the invasion

of microbes. Following successful wound healing, annelids proceed with the regeneration process based on two different mechanisms, namely epimorphosis and morphallaxis [19, 20]. In epimorphosis, re-establishment of tissue is initiated by the proliferation of undifferentiated cells to form blastema [16, 21]. On the other hand, morphallaxis is a compensatory form of regeneration, in which remodelling of tissue happens without notable stem cell differentiation [17, 21, 22]. The regeneration process involves several intricate molecular and cellular mechanisms, such as upregulation of specific genes and the activation of key transcription factors like HOX and Oct-4, which are regulated by different cellular signals for the successful completion of regeneration [23]. The comprehensive understanding of overall mechanism of regeneration in earthworms is significant not only to understand the key prospectives in the process of regeneration, but also in the field of ecology and its biomedical applications. The present review encompasses the key aspects of regeneration in earthworms, along with its facets of ecological implications.

MATERIALS AND METHODS

The present systematic review has been structured according to the Scale for the Assessment of Narrative Review Articles (SANRA) guidelines [24], incorporating the ecotoxicological and mechanistic dimensions of earthworm regeneration. The narrative and structure of the present review was formulated through an extensive search of the available scientific literature using the electronic databases, such as SpringerLink, Web of Science, Scopus, Google Scholar, ScienceDirect (Elsevier), PubMed and some additional preprint servers like bioRxiv. The relevant data and information were collected from these resources using various keywords and search prompts, such as "Earthworm regeneration," Blastema formation," "stem cells," "role of pluripotency factors in regeneration," "role of clitellum in earthworm regeneration," "genes and proteins involved in earthworm regeneration," ecotoxicological aspects of earthworm regeneration," "factors affecting earthworm regeneration," "impact of soil contaminants on earthworm regeneration," "regeneration gradient in earthworms," "mechanism of regeneration in earthworms," and others as per the requirement encountered during preparation of the present review. Additional searches were also conducted using the names of different earthworm species studied for regenerative biology or factors affecting their regeneration process, as well as with other terms, such as "transcriptome," "epimorphosis," "morphallaxis," "regenerative capacity" and "gene expression during regeneration in oligochaetes". The present review included the studies, which were published in peer-reviewed journals with authenticated indexing or in recognized preprints, and reported scientific data on the mechanism, molecular, cellular, physiological, ecological and ecotoxicological aspects of earthworm regeneration. The studies were excluded that were focused specifically on non-Oligochaeta with no relevance to earthworm regeneration; purely observational or ecological surveys, available only as conference abstracts without a peer-reviewed publication; and were not accessible in English. The references were managed using Mendeley cite PC version.

Mechanism of Regeneration in Earthworms

Earthworm do possess a gradient of regeneration capability along their body length. Martinez et al., [25] have reported that the anterior and posterior regeneration sites reconstruct a considerably different series of organs. Irrespective of these differences, the regeneration procedure into both anterior and posterior sites can be classified into five stages:

Wound healing

Wound healing is the initial step in the regeneration process of earthworms, primarily involving the skin tissues. The skin is the largest organ, protecting the host from the external environment and maintaining homeostasis [26]. Wounds create entry points for external materials and organisms into the body, and therefore healing of wounds is a complex, coordinated, and intricate biological process that involves inflammation, proliferation, and remodelling phases [27]. Immediately after the cut, the wound site is sealed by an epidermal cap, followed by the extension of epiblasts (undifferentiated cells) across the surface to make a sheath over the wound [20]. Specific immune cells, such as

coelomocytes, also play a key role in wound healing during regeneration, demonstrating the wound-triggered innate immune response [28], [29]. According to Baveja [30], wound covering can occur through two mechanisms: alimentary canal inversion and coelomic plug formation. Previous studies reported that muscle contraction plays a role in wound closure in the earthworm *Dendrobaena veneta* [31, 32]. According to previous reports, understanding the molecular mechanisms involved in wound healing could lead to a better interpretation of the conserved genomic processes underlying regeneration.

Blastema formation

During regeneration, the cells beneath the epidermal cap undergo the process of dedifferentiation to regain their embryonic stem cell properties and forms a mass of cells typically known as blastema. Park et al., [12] reported that the blastema formation during tail regeneration in earthworms represents an epimorphic model, and the longitudinal muscle layer of the body wall serve as the major site of origin of blastemal cells (Fig. 1A, B).

Three hypotheses have been proposed to explain oligochaete regeneration, but despite 130 years of supposition, conclusive evidence remains elusive. These theories propose the presence of multipotent neoblasts, migration of cells to the amputation site for blastema formation, and cell migration along the ventral nerve cord (VNC) to the blastema. Recent findings indicate that germ cell precursors accumulate along the VNC towards the anterior blastema. Although progress has been made in identifying molecules involved in blastema formation, these hypotheses still require clear validation [33]. Soon after the wound closes, nerve fibres from the ventral nerve cord and nearby peripheral nerves grow into the injury site (Fig. 1C). At the same time, cell division increases, especially in the epidermis and stomach. Many of these dividing cells push through the epidermis and form a cluster of pales, undifferentiated cells called the blastema (Fig. 1E). Early researchers (Randolph, [34]; Von Wagner [35]; Sayles [36]) reported that large cells called neoblasts move to the wound, divide there, and help form the blastema (Fig. 1D, F, H). However, other studies, particularly on anterior regeneration, did not find evidence for neoblast migration. During this stage, muscle cells often lose their normal fibrous structure and detach into the coelomic cavity as free-floating myocytes (Fig. 1E) [25].

During regeneration, various tissues, including myofibers, satellite cells, cartilage, dermis, and nerve cells, contribute to blastema formation following amputation. Blastema formation is a critical step in regeneration, enabling the restoration of lost structures through subsequent differentiation. Studies of early wound healing, particularly the work of Park et al., [12] reported that immediately after injury, the intestine is extruded and subsequently covered by an epithelial layer during the first 3÷5 days. By day 7, a small blastema composed of undifferentiated cells becomes evident. This blastema arises through dedifferentiation of tissues adjacent to the injury site, with longitudinal muscles on the coelomic side contributing most significantly. As blastema cells proliferate and recruit additional cells, extracellular matrix production increases, resulting in noticeable tissue swelling. Segmentation of the blastema begins beneath the wound dermis within the first week, although substantial redifferentiation is not initiated until later. Between days 14 and 17, the blastema enlarges and produces visible regenerative outgrowths. Throughout the first three weeks, the regenerating tissue remains noticeably lighter in color than the mature segments. Rapid growth and pigmentation become apparent around day 26, and by approximately 30 days post-amputation, the intestine reopens, allowing the worm to resume feeding and excretion [37].

Blastema differentiation

The blastema is typically formed from the clonogenic neoblasts, cells of the longitudinal muscle layer of the body wall, and in some cases, the clitellar cells also take part in blastema formation [38, 39]. Rapid development and proliferation of this blastema is followed by segmentation and gradual redifferentiation into new tissues, such as muscle, nerve, and skin, which can be seen as outgrowths and ultimately result in the restoration of the lost body part [40]. In a detailed study by Martinez et al., [25], the blastema formation and differentiation have been considered as a coordinated program

of differentiation that establishes the non-segmental terminal structures and sets the foundation for subsequent segment formation. During this phase, the blastema begins to develop into the worm's terminal end regions: in anterior regeneration, it differentiates into a cone-shaped prostomium (Figures 1E, G), whereas in posterior regeneration, it gives rise to a pygidium bearing a newly formed anus (Figures 1F, H).

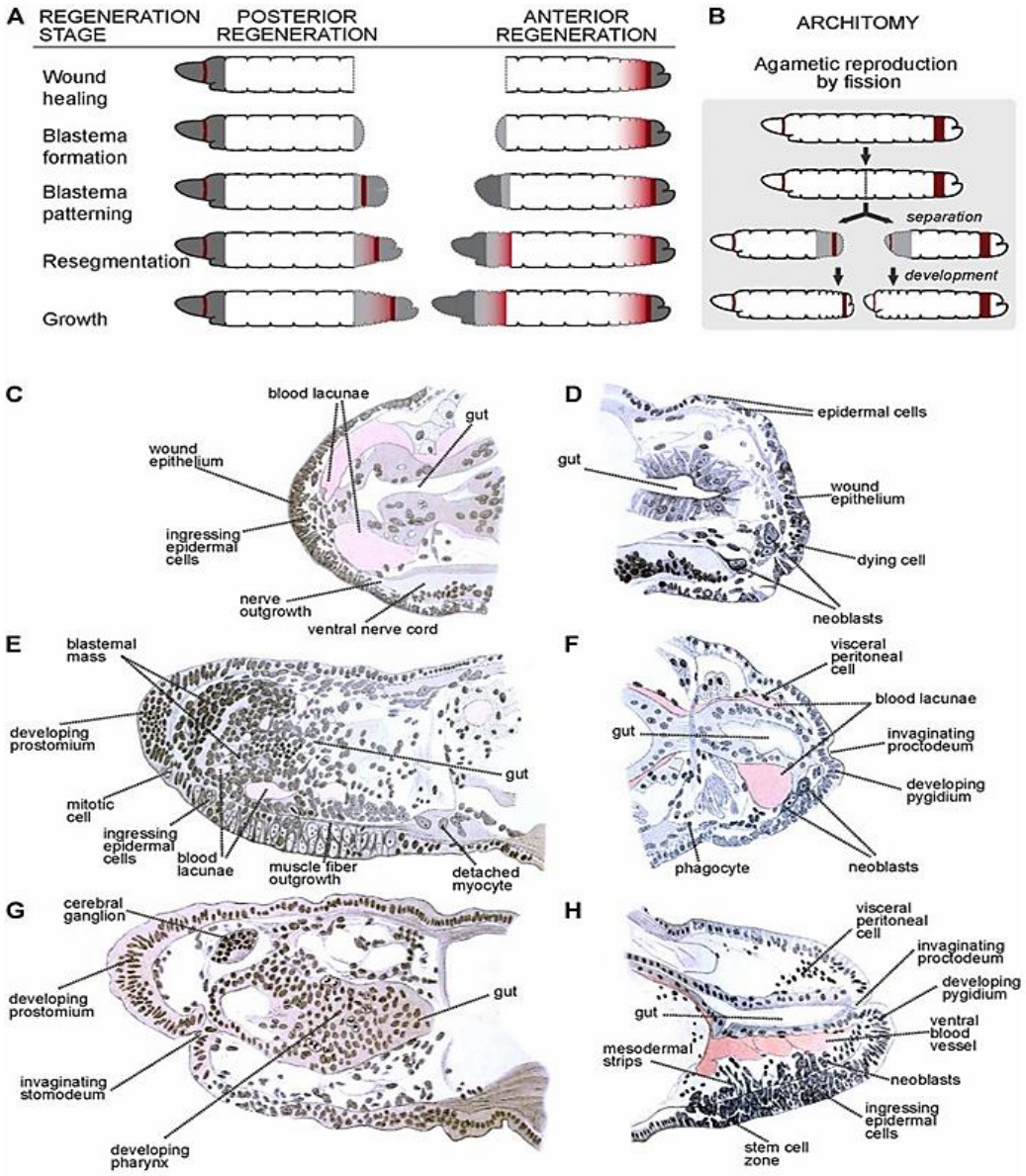

Fig. 1. Regeneration and asexual reproduction in *Lumbriculus*. (A) Generic stages of annelid regeneration. Dashed line: cut/regenerated tissue; dark gray: non-segmental tissues; dark red: mitotically active areas; gray shading: differentiating segmental tissues. (B) Asexual reproduction by fission. Coloring as in A. (C–H) Histological sections through early (C), middle (E) and late (G) anteriorly regenerating individuals, and early (D), middle (F) and late (H) posteriorly regenerating individuals. (C–H) [25].

Re-segmentation

In earthworm regeneration, re-segmentation is the specific stage where the mass of undifferentiated cells, known as the blastema, organises to form new, discrete body segments and associated internal structures. Segmentation in earthworm regeneration is characterised by a gradual increase in the number of segments in the newly forming region, typically seen within a few days after an amputation [37]. During this stage, the blastemal mass splits into more specific clusters of dorsal, lateral, and ventral cells. Chetal sacs are formed by the dorsal and lateral clusters, exuding locomotory chaetae

[41]. The ventral clusters comprise the nerve cord ganglia. The brain has completed its differentiation, and circular muscle fibres create fine rings between the epidermis and the longitudinal muscle. As with normal growth, the regeneration begins to form additional segments at its new posterior growth zone [25].

Growth

The growth phase represents the final stage of regeneration, where newly formed tissues undergo maturation, expansion, and functional integration. Following re-segmentation, regenerated organs and segments progressively enlarge to attain the proportions of intact tissues. Cellular proliferation, extracellular matrix remodelling, and pigment deposition contribute to structural refinement. Functional recovery includes reopening of the alimentary canal, restoration of locomotory chaetae, and re-establishment of neuromuscular coordination. This stage ensures that regenerated regions are physiologically indistinguishable from original segments, enabling feeding, reproduction, and locomotion. Growth thus consolidates regeneration by harmonising morphology and function [25, 37].

Molecular mechanisms involved in regeneration

Regeneration in earthworms involve precise and specific cellular and molecular mechanisms to regenerate different types of cells or tissues from pluripotent or potentially totipotent blastemal cells [42]. The molecular mechanisms underlying earthworm regeneration implies a precisely controlled and modulated system of various transcription factors, specifically controlled and regulated expression of certain genes and cellular programming pathways, which together lead to wound healing, cell proliferation, differentiation, tissue-patterning and finally organogenesis. Immediately after injury the wound-healing initiates, followed by the activation of stress and immune- response pathways and specific molecular signalling cascades, such as Wnt/β-catenin, Notch, Hedgehog, FGF, and BMP pathways, which play key roles in the regeneration process [43÷45].

Genes involved in earthworm regeneration

In earthworms, the mechanism of regeneration encompasses several intricately related and complex mechanisms entailing cellular reprogramming, dedifferentiation and redifferentiation, driven by upregulation of specific pluripotent factors and specific gene families [43, 45]. Approximately 622 Expressed Sequence Tags (EST), or roughly 338 genes, were implicated in regeneration, according to a transcriptomic study [42]. Zhang et al. [46] reported that the upregulation of three labial genes (Pex-lab01, Pex-lab02, and Pex-lab03) and the distal-less gene induce head regeneration in *Eisenia fetida*, and dedifferentiation and reprogramming are regulated by the upregulation of five pluripotent factor genes (Sox2, Oct4, Nanog, Lin28, and c-Myc). Furthermore, throughout the regeneration process, five superfamilies of Sox, Pax, Wnt, Klf, and Hox show higher expression, with the Pax gene aiding in bud/blastema formation, and the Wnt gene stimulating various cellular signalling pathways involved in body axis formation. These six superfamilies are believed to promote bud formation and the remodeling of blood vessels, fats, nerves, and muscles during regeneration in *Eisenia fetida* [46]. Numerous studies have reported the transcriptome profiles of various earthworm species during regeneration to better understand the expression of certain genes that are upregulated during this process. Rossan Mathews *et al*. [47] studied the transcriptome of *Perionyx excavates* during anterior epimorphic regeneration. Paul *et al*. [48] reported the transcriptome profile of anterior regeneration in the earthworm *Eudrilus eugeniae* using RNASeq, identifying 10,868 differentially expressed genes (DEGs), of which 3986 genes were significantly upregulated in the anterior regenerating blastema. Sahu *et al*. [45] reported the expression of 239 genes in the regenerated tissues of earthworms *Drawida calebi* and 241 genes in *Lampito mauritii*. Most of these genes were associated with neuronal regeneration, cell signalling, immune response, cytoskeleton maintenance, and resistance to oxidative stress. Shao *et al*. [49] highlighted the crucial role of EGFR signalling, LINE2 transposable elements, and early growth response genes in the regeneration process of *Eisenia andrei*. While the transcription profile of *Eisenia fetida*, an exotic species, has been documented during regeneration

[46], in Indian contexts, there is a scarcity of research on the transcriptome profiles of many native earthworms, which are commonly found in both cultivated and uncultivated soils. Stem cell pluripotency factors include genes involved in embryonic stem cell (ES) pluripotency and cellular differentiation, such as octamer-binding transcription factor 4 (Oct4); a transcription factor (Nanog); sex-determining region of Y chromosome-related high mobility group box 2 (Sox2); kruppel-like factors 4 (Klf4); a microRNA-binding protein (Lin28); and proto-oncogenes like c-Myc, a cellular gene similar to myelocytomatosis viral oncogene [50].

Based on several studies to date (Zheng et al., [46]; Bhambri et al., [43]; Tao et al., [51]; Shao et al., [49]; Subramaniam et al., [44]), several genes, gene families, and pluripotency factors involved in earthworm regeneration can be broadly categorized into various groups. The pluripotency factors include Oct4, Sox2, Lin28, Nanog, and C-myc, among others, which are significantly upregulated during blastema formation, playing key roles in cellular reprogramming. Signalling and transcription factors include Pax (involved in blastema formation), Wnt (body axis formation), Hox (body segmentation), and Sox superfamily genes. Differentiation factors include Sox4 (involved in epithelial-mesenchymal transition) and nerve growth factor, involved in the repair of the nervous system. Several genes, such as Pex-ADAR and others, are involved in redifferentiation and muscle regeneration. The growth factors, such as EGFR (Epidermal Growth Factor Receptors), also play a key role in cell proliferation during regeneration. Some of the genes reported to play key roles during earthworm regenerations include:

Y Chromosome related high mobility group 2 (Sox2) gene

The Sox2 gene plays a pivotal role in cellular reprogramming during head, middle, and tail regeneration, and encodes a transcription factor essential for maintaining pluripotent stem cell populations and guiding differentiation during early development [52]. Members of the Sox family are widely expressed in developing tissues, regulating cell proliferation, lineage specification, and stem/progenitor cell establishment [53]. Sox2 is a critical pluripotency factor in *Eisenia fetida*, enabling somatic cells to dedifferentiate into blastema stem cells following injury. Its mRNA expression rises sharply within hours, in coordination with other pluripotency genes such as Oct4, Nanog, Lin28, and c-Myc [52]. This transcriptional surge maintains stemness, drives blastema formation, and subsequently directs the differentiation of new cells into diverse tissues, including nerves, muscles, blood vessels, and connective structures. Sox2 expression peaks during regeneration after amputation, and its function is significantly inhibited by toxins such as retinoic acid, underscoring its essential role in blastema formation and tissue repair [54].

Octamer binding transcription factor 4 (Oct4) gene

Oct4 is a pivotal pluripotency factor implicated in the regenerative capacity of *Eisenia fetida*. Research demonstrates its upregulation alongside Sox2, Nanog, Lin28, and c-Myc during the early phases of cell reprogramming and dedifferentiation, enabling somatic cells to revert to a stem-cell-like state and contribute to blastema formation [46]. Oct4 expression peaks approximately 12 hours post-amputation, suggesting a role in initiating regeneration, though its temporal dynamics differ from other factors, such as Nanog, which peaks later [52]. Interestingly, some studies report Oct4 expression in reproductive tissues, particularly ovaries, with comparatively lower levels during regeneration, highlighting its context-dependent function [46]. Overall, Oct4 operates within a complex, multi-gene regulatory network that orchestrates earthworm regeneration and supports the formation of new body structures.

Myelocistomatosis viral oncogene (c-Myc)

The c-Myc gene is an oncogenic transcription factor that plays a central role in cell cycle progression, apoptosis, and cellular transformation, by regulating cellular proliferation, dedifferentiation and cell reprogramming [55]. In normal somatic cells, c-Myc expression is low, but it is essential for maintaining proliferation [56]. It plays a key role in enabling the normal somatic cells to regain their pluripotent properties [46]. In earthworms, c-Myc expression is absent in head and tail tissues but

higher in the clitellum compared to cocoons, indicating increased mitotic activity in reproductive tissues. During regeneration, c-Myc shows two distinct expression waves, remaining relatively high even six days post-amputation, unlike other pluripotency factors that decrease. This persistent expression suggests ongoing mitotic activity essential for blastema expansion and tissue remodelling [37, 57, 58].

Micro RNA binding protein coding gene (Lin28)

The Lin28 gene plays a crucial role in the regenerative capacity of *Eisenia fetida*, functioning as a pluripotency factor that promotes cellular reprogramming and dedifferentiation necessary for regenerating lost body parts [46]. After amputation, Lin28 expression is significantly increased, mirroring other stem cell markers like Oct4, Nanog, and Sox2. This increase allows mature somatic cells to revert to a primitive, stem-like state, aiding blastema formation and rapid tissue growth. Mechanistically, Lin28 binds to and blocks the let-7 microRNA, a key regulator in development, thus maintaining the expression of genes needed for proliferation and pluripotency. Additionally, Lin28 promotes metabolic reprogramming by increasing translation of enzymes involved in glycolysis and oxidative phosphorylation, ensuring a sufficient energy supply for regeneration [59]. In combination with Oct4, Sox2 and Nanog, it enhances the efficiency of reprogramming during regeneration [46]. Empirical studies in *Eisenia foetida* show that Lin-28 mRNA levels increase hundreds of times within days post-injury, emphasising its active role in the regeneration process. Conserved across species, Lin28 also encourages tissue repair in mammals, underscoring its broad biological significance [46, 60]. Table 1 summarizes the key genes identified in different earthworm species, highlighting their specific roles in pluripotency, blastema formation, tissue differentiation, and axis patterning during regeneration.

Table 1. Genes involved in earthworm regeneration and their functions.

Genes	Earthworm species	Function	Reference
Sox2 gene	*Eisenia fetida*	Maintains pluripotency of stem cells; regulates dedifferentiation during blastema formation and directs tissue-specific differentiation (nerve, muscle, epidermis). Upregulated within hours post-amputation.	[52], [53]
Oct4 gene	*Eisenia fetida*	Key pluripotency transcription factor initiating dedifferentiation of somatic cells to blastemal stem cells; works synergistically with Sox2 and Nanog during regeneration.	[52], [59]
c-myc gene	*Eisenia fetida*	Regulates cell proliferation and blastema expansion; maintains mitotic activity up to 6 days post-injury, essential for rapid tissue remodelling.	[37], [57]
lin28 gene	*Eisenia fetida*	Enhances cellular reprogramming and inhibits *let-7* microRNA to sustain pluripotency; promotes metabolic reprogramming during tissue regeneration.	[46], [60]
Hox genes (Pex-lab01, Pex-lab02, Pex-lab03)	*Eisenia fetida*	Control anterior–posterior body patterning and regulate blastema formation; essential for head regeneration.	[37], [48]
Wnt family (Wnt3a)	*Eudrilus eugeniae*	Activates canonical Wnt signalling for cell polarity and axis formation during blastema differentiation.	[40], [61]
Pax gene family	*Eisenia fetida*	Promotes bud/blastema formation and regulates stem cell migration during regeneration.	[46]
Nanog	*Eisenia fetida*	Maintains pluripotency state of blastemal cells; peaks later than Oct4 during regeneration cascade.	[46], [52]

Proteins involved in earthworm regeneration

Different stages of regeneration in earthworms involves specific set of proteins, responsible for activation of stem cells, cell proliferation, and other key mechanisms during dedifferentiation, redifferentiation and organogenesis. Some of the major proteins playing key role in earthworm regeneration include Translationally controlled tumour protein (TCTP), Sox2 protein, Wnt3a, Proliferating cell nuclear Antigen (PCNA), EGF, YAP1, and HoxD3, among others [40]. Few of these functionally important proteins are discussed below:

TCTP Protein

The Translationally Controlled Tumour Protein (TCTP) is a multifunctional protein, conserved across species from worms to humans. TCTP regulates cell proliferation, differentiation, apoptosis, anti-apoptosis, stem cell maintenance, and immune responses, although only limited studies have directly linked it to regeneration [40]. In *Eudrilus eugeniae*, the highest TCTP expression was observed at the blastema tip during posterior body regeneration. Silencing TCTP with siRNA blocked regeneration, delayed wound healing, and inhibited epithelial granular cell differentiation and migration from the clitellum, confirming its vital role in regenerative processes [39].

Wnt3a

This protein plays crucial role in wound healing, promoting blastema formation through stem cell activation and in other pathways routed into the signalling mechanisms and regulation of regeneration [62] . It is a member of the Wnt ligand family, and an important marker for stem cells, playing a crucial role in the initiation of tissue restoration through epimorphosis and morphallaxis. Wnt3 serves key functions, such as activation and maintenance of stem cells, anterior-posterior axis specification, cell proliferation and migration, and wound healing [61]. One of the most crucial functions of Wnt3a protein includes its role in maintenance of anterior-posterior polarity, which functions as a positional information assuring the regeneration of either head or tail segments in the correct orientation [63]. It also plays a key role in the activation of dormant stem cells and maintenance of their stemness during the regenerative process apart from accelerating the wound closure, reducing inflammation and facilitating the epithelium renewal to maintain the integrity of amputation site before the start of complex tissue restoration [40].

EGF-protein

EGF-like proteins, such as fibropellin and Notch receptors, among others, play a pivotal role in earthworm regeneration by regulating cell proliferation, differentiation, and wound healing. The EGFR gene family shows remarkable expansion in earthworms, with *Eisenia andrei* possessing up to 12 copies compared to 0-2 in other species, underscoring its importance in regeneration [49]. EGFRs, as transmembrane receptors with tyrosine kinase activity, activate signalling cascades essential for regrowth after injury [64]. Earthworm extracts containing EGF-like domains (e.g., G-90, PvE-3) bind strongly to EGFRs, accelerating wound healing in mammalian models by stimulating fibroblast and epithelial proliferation. Additionally, clitellum-derived factors modulate EGF pathways, influencing stem cell activity and tissue remodelling [49]. It has also been reported that the expression of EGF like proteins initiates early after amputation and return back to controlled levels 12 hrs post-amputation [37].

DNA- binding proteins

The DNA-binding proteins serves as crucial regulators of transcription of the genes which are primarily involved in redifferentiation of cells and reorganization of the damaged tissue [37]. DNA-binding proteins act as transcriptional regulators, initiating developmental processes where undifferentiated cells differentiate into specialized cell types. Earthworms regenerate by dedifferentiating into pluripotent stem cells, with pluripotency factors such as Oct4 at the core of the transcriptional hierarchy controlling self-renewal and embryonic pluripotency. Additionally, the c-Myc gene family, known as oncogenic transcription factors, plays a crucial role in maintaining the

cell cycle and apoptosis in both somatic and cancer cells, and is similarly vital in regenerative processes [37]. Table 2 highlights the major proteins that regulate stem cell activation, cell proliferation, dedifferentiation, and morphogenesis during different stages of regeneration in earthworms.

Table 2. Proteins involved in earthworm regeneration and their functions.

Protein	Earthworm species	Function	Reference
TCTP Protein	*Eudrilus eugeniae*	Regulates proliferation, apoptosis inhibition, and wound healing; silencing delays epithelial differentiation and blastema formation.	[40, 62]
Sox2protein	*Eisenia fetida*	Transcription factor maintaining stem cell pluripotency; promotes dedifferentiation and controls timing of blastema development.	[46, 52]
EGF-like Protein / EGFR	*Eisenia andrei*	Stimulates cell proliferation, epithelial healing, and stem cell activation via EGF–EGFR signaling; highly expanded EGFR family in earthworms.	[49]
DNA-binding protein (e.g., Octamer-binding transcription factor/Myc gene family)	*Eisenia fetida*	Regulate transcription and differentiation; coordinate activation of pluripotency network for tissue regrowth.	[37]
H2AX	*Eisenia fetida*	DNA repair-associated histone variant that maintains genomic stability in dividing blastema cells.	[40]

Role of clitellum in regeneration

Despite their morphological similarities, the regenerative capacities in earthworms vary significantly among species, primarily due to differences associated with the clitellum, a specialized glandular structure found in mature individuals [65]. The clitellum plays a dual role in both reproduction and regeneration, acting as a biological hub of stem cells and molecular signalling factors that govern tissue renewal [40, 62]. In clitellum-dependent species such as *Eudrilus eugeniae*, regeneration is strictly contingent upon the presence of intact clitellar segments. Amputated segments lacking the clitellum fail to regenerate, underscoring its indispensable role in providing stem cell populations and regeneration-promoting bioactive factors [20, 44]. Recent transcriptomic and proteomic studies have demonstrated that the clitellum secretes several regenerative proteins and transcriptional regulators, including Translationally Controlled Tumor Protein (TCTP), Wnt3a (Wingless type MMTV integration site family member 3A), YAP1 (Yes Associated Protein 1), and VEGF (Vascular Endothelial Growth Factor), all of which coordinate cellular proliferation, blastema formation, and organogenesis [40]. These factors activate epimorphic and morphallactic pathways, enabling dedifferentiation and reprogramming of somatic cells into pluripotent stem cells.

Furthermore, the clitellum acts as a stem cell reservoir, releasing molecular cues that enhance wound healing, tissue remodelling, and axis formation [61]. In contrast, clitellum-independent species such as *Perionyx excavatus* exhibit remarkable regenerative abilities even in the absence of clitellar tissue, suggesting evolutionary divergence in the control of regenerative mechanisms [44, 45]. Collectively, the clitellum's multifaceted functions as a source of pluripotent cells, regulatory proteins, and signalling factors position it as a central organ in the regeneration biology of annelids, providing a unique model for studying stem cell-mediated regeneration and tissue engineering. In recent times, numerous studies have been conducted using the earthworm model to explore various areas. In this context, the different roles of clitellum in regulating and controlling stem cells, the regeneration process, regulation of organogenesis, stress response, ageing, autotomy, and various features have been briefly discussed.

Clitellum as a source of stem cells in clitellum-dependent worms

The mechanisms of regeneration in earthworms can be broadly classified into two categories: clitellum-dependent and clitellum-independent [18]. In clitellum-dependent species such as *Eudrilus eugeniae*, successful regeneration requires the presence of intact clitellar segments, whereas clitellum-independent species like *Perionyx excavatus* retain regenerative capacity even in the absence of the clitellum, presumably through alternative stem cell niches or localised morphogen gradients [44, 45]. Amputation experiments in *E. eugeniae* demonstrate that fragments containing the clitellum regenerate complete body structures, while clitellum-free segments exhibit wound healing without true regeneration, underscoring the clitellum's essential regulatory role [20].

The clitellum functions as a specialized stem cell niche and a molecular signalling hub, providing both pluripotent cells and bioactive regulatory factors that orchestrate dedifferentiation, blastema formation, and redifferentiation at the wound site. It secretes key proteins and growth factors, including Translationally Controlled Tumour Protein (TCTP), Wnt3a, YAP1, VEGF, and Caspase-3, which collectively regulate cell proliferation, survival, stress response, wound healing, and tissue patterning [20, 40]. Transcriptomic analyses reveal that anterior regeneration is accompanied by the upregulation of approximately 3,986 genes, many under the control of clitellum-associated signaling pathways, with the clitellum displaying higher transcriptional activity than head or tail regions [40, 46, 49].

Role of clitellum in organogenesis

The clitellum not only initiates regeneration but also governs organ differentiation and morphogenesis. In *E. eugeniae*, amputated segments lacking a clitellum can form blastema-like structures, but fail to undergo full organogenesis. Only in the presence of clitellum-derived factors can the blastema differentiate into complex organs such as the mouth, brain, heart, seminal vesicles, and ovaries, primarily located in the anterior region [18, 40]. When clitellum activity is lost or diminished, differentiation signals decline, leading to incomplete or defective regeneration. This observation aligns with the anterior preference for regeneration observed across annelids, since anterior regions contain critical organs and neural structures, they receive priority in molecular signalling and resource allocation during regeneration [25, 45]. Therefore, the clitellum not only contributes to regenerative initiation but also modulates the fidelity of organogenesis through the release of hormones, growth factors, and transcriptional regulators.

Regeneration capacities in different earthworm species

Within annelids, earthworms (Oligochaeta) exhibit a wide spectrum of regenerative capacities, ranging from complete regeneration of the anterior and posterior ends to partial or no regeneration at all. These differences depend on species type, site of amputation, physiological condition, and clitellum dependency [25, 62]. The annelids represent an ideal model for studying the evolution of regeneration, as they encompass species with no regenerative ability as well as those capable of regenerating entire individuals from a single body segment [16, 44]. Most earthworm species exhibit posterior regeneration, i.e., restoration of the lost tail region, while anterior regeneration (head formation) is limited to a few taxa. Table 3 compares the regenerative abilities of selected earthworm species, highlighting differences in anterior and posterior regeneration and overall regenerative potential.

Table 3. Variations in regeneration capacities of different earthworm species

Earthworm species	Type of regeneration	Description and characteristics	References
Eisenia fetida	Posterior and limited anterior	Capable of regenerating both tail and limited head segments. Anterior regeneration depends on the amputation level; regeneration decreases posteriorly beyond segment 23. Exhibits active gene expression of pluripotency factors (*Sox2*, *Oct4*, *Nanog*) and signaling pathways (*Wnt*, *Pax*).	[46, 49, 66]

Earthworm species	Type of regeneration	Description and characteristics	References
Lumbricus terrestris	Posterior only	Commonly used model in soil biology; regenerates tail segments effectively but fails to regenerate head beyond mid-body amputation. Growth rate and survival decline in pesticide-contaminated environments.	[7, 67]
Eudrilus eugeniae	Clitellum-dependent anterior and posterior	Exhibits strong regeneration when clitellum is intact; amputated segments without clitellum fail to regenerate. Regeneration controlled by clitellar stem cell factors including *TCTP*, *YAP1*, and *Wnt3a*.	[40, 62]
Perionyx excavatus	Bidirectional (anterior & posterior)	Highly regenerative, clitellum-independent species capable of regenerating entire anterior structures (brain, mouth, gonads) within 25 days. Shows epimorphic regeneration with rapid blastema formation and organogenesis.	[44, 45]

Modes of regeneration in earthworms: Epimorphosis and Morphallaxis

Earthworms employ two principal regenerative modes- epimorphosis and morphallaxis, each governed by distinct cellular and molecular mechanisms. Epimorphosis, the predominant mode in *E. eugeniae* and other annelids, involves the proliferation of dedifferentiated stem cells to form a blastema that subsequently differentiates into lost tissues and organs. Histological studies indicate that stem cells originating from the circular muscle layer migrate to the longitudinal muscle zone beneath the wound site, where blastema formation occurs. This migration is accompanied by thinning of the circular muscle layer and thickening of the longitudinal layer, providing structural support to the regenerating tissue [12, 40]. This process is regulated by pluripotency-associated genes such as Sox2, Oct4, and Nanog, which are upregulated during cellular dedifferentiation [46]. In contrast, morphallaxis involves tissue remodelling without blastema formation and relies on the reorganization and trans differentiation of existing tissues to restore positional identity and lost structures. This mode is characterized by the trans-differentiation of multipotent epithelial cells, particularly within the atrial epithelium, and has been documented in *E. eugeniae* and *Lumbricus terrestris* [39]. Notably, regression of clitellar segments following regeneration suggests active migration of clitellum-derived cells toward the injury site [18]. Central to both epimorphic and morphallactic regeneration is TCTP, which plays a critical role in stem cell activation, cellular stress tolerance, and survival signalling. Experimental suppression of TCTP using buclizine results in delayed regeneration, impaired wound closure, and reduced expression of PCNA, Wnt3a, and YAP1, providing direct evidence of its indispensable role in regenerative control [40].

Factors affecting regeneration in earthworms

The factors modulating regenerative processes in earthworms can be delineated across multiple domains, reflecting the interplay of intrinsic biological mechanisms, extrinsic environmental conditions, and molecular regulatory pathways:

Site of amputation and regeneration gradient along the body

The site of amputation is a fundamental determinant of regenerative success in earthworms, influencing both survival rates and the extent of tissue regrowth. The regenerative capacity varies along the anterior-posterior body axis, with anterior segments exhibiting greater regenerative potential than posterior ones. Experimental research on *Eisenia fetida* demonstrated that worms amputated closer to the anterior end (segments 6-7) showed progressive and complete regeneration, forming distinct blastemal tissue and successfully restoring anterior organs such as the prostomium and nerve ganglia (Type I regeneration). In contrast, individuals amputated beyond the 23rd segment, retaining less than one-fourth of their body length, exhibited incomplete regeneration (Type II), often limited to wound closure or partial tissue remodelling [25, 66]. Further analysis revealed that survival rates were more closely correlated with the number of remaining body segments rather than the

amputation position itself. Worms with a greater number of intact segments demonstrated enhanced metabolic stability and regenerative potential, while severely truncated individuals experienced lower survival and slower wound recovery. Notably, juvenile (non-clitellate) earthworms displayed superior regenerative ability compared to clitellate adults, suggesting that cellular plasticity and mitotic activity decline with sexual maturity [18, 66]. This phenomenon parallels the stem cell exhaustion model observed in higher organisms, where the differentiation potential decreases with age and metabolic specialization.

The regeneration gradient hypothesis postulates that regenerative potential progressively declines from the anterior to the posterior ends of the body. The anterior region, rich in neural and stem cell clusters, exhibits higher cellular totipotency and stronger activation of pluripotency-related genes, whereas the posterior region regenerates more rapidly but produces simpler tissues, such as muscle and intestine. This polarity is regulated by conserved molecular signaling pathways, particularly Wnt, Hox, and Sox gene families which establish segmental identity, axis formation, and tissue patterning during regeneration [40, 49]. Collectively, these findings highlight that regeneration efficiency in earthworms is a gradient-driven process modulated by anatomical position, developmental stage, and gene regulatory dynamics [20]. The positions of *Eisenia fetida* amputations and their corresponding regenerative outcomes are illustrated in Figure 2 [66].

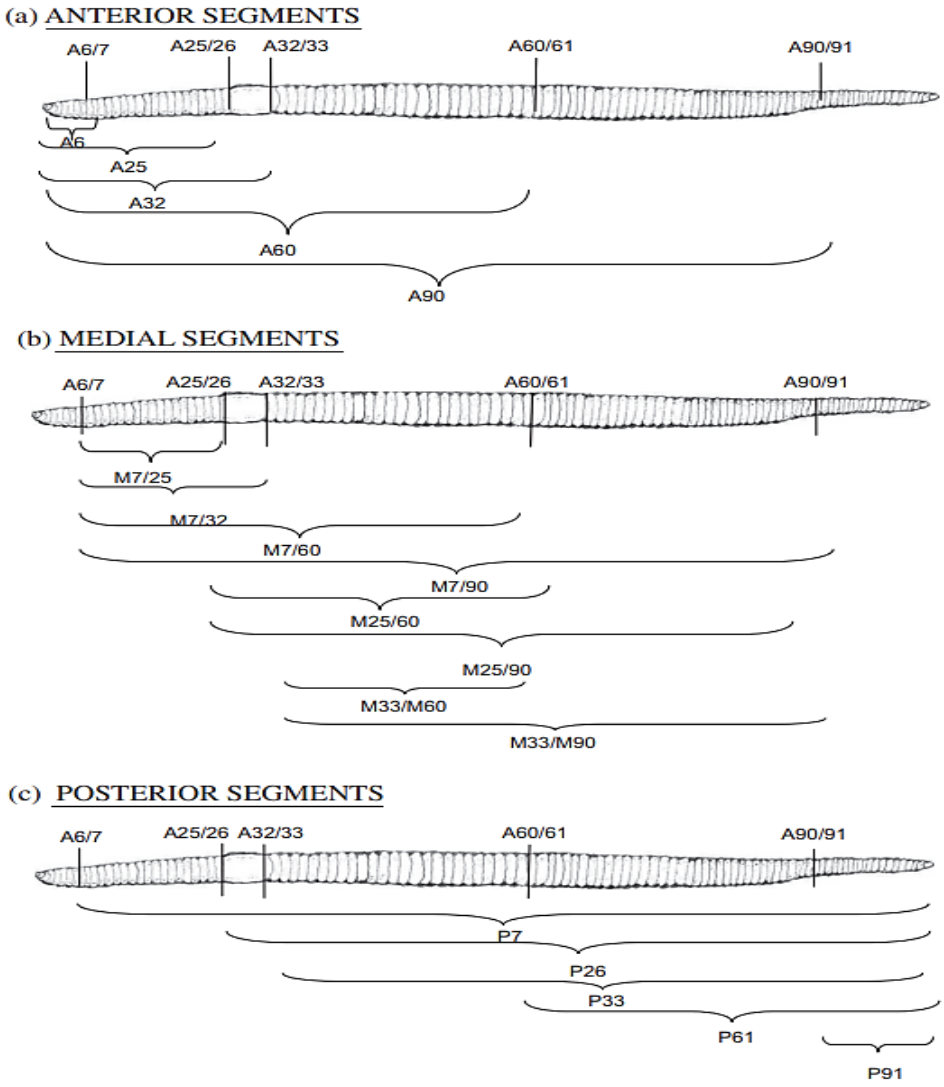

Fig. 2. Sites of amputation in Eisenia fetida and their regenerative outcomes. This figure depicts the experimental positions of anterior and posterior amputations. Anterior amputations (segments 6–7) resulted in complete regeneration of prostomium and nerve ganglia (Type I), whereas posterior amputations beyond the 23rd segment led to incomplete regeneration, limited to wound closure or partial tissue remodeling (Type II). (Adapted from Xiao *et al*. [66])

Physical and Chemical factors

Physical Factor

The regeneration capacity of earthworms is profoundly influenced by physical environmental factors, which affect both their physiological homeostasis and cellular repair mechanisms. According to Capowiez *et al*. [68], earthworms contribute significantly to the restoration of compacted soils, enhancing soil structure, aeration, and fertility through their burrowing and casting activities. In reduced or minimum-tillage agricultural systems, promoting earthworm density and activity is crucial, even though complete soil regeneration by their biological action is a gradual ecological process.

Several interrelated physical parameters, including temperature, moisture, soil pH, aeration, soil composition, organic matter content, and food availability, directly affect earthworm survival, growth, and regenerative efficiency [69]. These variables also determine the rate of blastema formation, tissue differentiation, and metabolic activity post-amputation.

Temperature

Temperature plays a critical role in modulating metabolic rate and cellular activity. Optimal regeneration occurs between 20°C and 28°C, while temperatures below 10°C or above 30°C significantly slow blastema formation and increase mortality rates [16]. Fluctuations in temperature affect enzymatic reactions, cell proliferation, and the stability of regeneration-related proteins.

Moisture

Adequate soil moisture is essential for maintaining coelomic fluid pressure and preventing desiccation stress. Moisture supports oxygen diffusion through the skin and enhances metabolic reactions necessary for wound healing and tissue repair. However, excessive waterlogging can reduce oxygen availability, leading to hypoxia and reduced regenerative potential [69].

Soil composition and aeration

Soil texture and aeration directly influence the physical environment of earthworms. Compacted soils reduce macroporosity and limit water infiltration, which negatively impacts earthworm biomass, reproduction, and regenerative capacity [68]. Loamy soils with high organic matter promote better respiration and nutrient cycling, improving regeneration outcomes.

Nourishment and organic matter

The availability of organic matter and food sources (e.g., decomposing leaves, microorganisms) enhances regeneration by providing essential nutrients and energy for cellular proliferation. A high level of soil humus increases microbial activity, which supports coelomic fluid composition and stem cell function [69].

Physical injury and stress conditions

Repeated physical injuries (e.g., predation, handling, or cultivation) can trigger localized regenerative responses but may also cause systemic stress that inhibits complete tissue recovery. Environmental stresses such as extreme dryness, pollutants, or vibration can elevate oxidative stress markers and disrupt coelomocyte activity, impairing the regenerative process [29].

Collectively, these physical factors interact to shape the regeneration efficiency and survival potential of earthworms. Maintaining stable soil conditions with adequate moisture, aeration, and organic matter is therefore vital for both soil ecosystem restoration and the biological regenerative performance of earthworm populations. Table 4 outlines how different physical conditions influence the regenerative capacity of earthworms, affecting wound healing, blastema formation, and overall recovery.

Table 4. Impact of various physical factors on earthworm regeneration

Factors	Earthworm species	Effect	Reference
Temperature	*Eisenia fetida*, *Lumbricus terrestris*	Optimal regeneration occurs at 20–28 °C. Low temperatures (<10 °C) slow blastema formation, while high temperatures (>30 °C) inhibit cell proliferation and increase mortality.	[16, 69]
Moisture	*E. fetida*, *E. eugeniae*	Adequate soil moisture maintains coelomic pressure, supports oxygen diffusion, and promotes wound healing; excessive waterlogging induces hypoxia and slows regeneration.	[69]
Soil Composition & Aeration	*Aporrectodea caliginosa*, *L. terrestris*	Compacted soils reduce porosity and regeneration; loamy soils with higher organic matter enhance nutrient cycling and tissue recovery.	[68, 69]
Soil pH and CO_2 Balance	*E. fetida*	Neutral to slightly alkaline pH (6.5–7.5) favors metabolic enzyme stability and cell proliferation; acidic conditions inhibit regeneration enzyme systems.	[69, 70]
Organic Matter & Nourishment	*E. fetida*, *P. excavatus*	Abundant organic matter increases microbial activity, nutrient availability, and stem-cell viability, accelerating blastema development.	[71, 72]
Physical Injury & Stress Conditions	*L. terrestris*, *E. andrei*	Mild injury stimulates localized regeneration, while repeated trauma or environmental stress (dryness, vibration, pollutants) induces oxidative stress, reducing coelomocyte activity.	[28, 29]
Oxygen Availability (Aeration)	*E. fetida*	Higher aeration improves epithelial healing and stem-cell activation; hypoxic conditions delay blastema formation.	[49, 68]

Chemical factors affecting regeneration

The chemical factors play a crucial role in the regulation of survival, growth and regenerative ability of earthworms. Exposure to pollutants or soil contaminants, such as pesticides, heavy metals, and industrial chemicals can adversely impact the physiological homeostasis, hinder cellular proliferation, and alter the expression of genes related to regeneration [73]. Previous studies have documented the adverse impacts of agrochemicals, such as pesticides, on biology of earthworms, inferring the growth rate and regeneration as sensitive biomarkers of environmental toxicity or contamination [73]. Xiao et al. [74]demonstrated that acetochlor, a chloroacetanilide herbicide, significantly suppresses growth, and can serve as a sensitive biomarker for toxicity assessment. Similarly, Helling et al. [75] observed detrimental effects of copper oxychloride, a commonly used fungicide, on the survival and metabolic activity of soil-dwelling annelids.

Yasmin *et al*. [76] further investigated the effects of carbendazim, glyphosate, and dimethoate on *Eisenia fetida*, and reported a significant concentration-dependent reduction in the growth rate. They also noted that pesticide exposure interferes with coelomic fluid composition and mitotic activity, leading to incomplete regeneration of amputated segments. Van Gestel et al. [77] found that parathion, an organophosphate insecticide, disrupts the normal growth and cellular turnover in *Eisenia andrei*.

In a comparative study, Booth et al. [78] evaluated the effects of two organophosphates chlorpyrifos and diazinon, and reported marked suppression in earthworm activity and blastema development. Mosleh et al. [67] tested the impact of multiple pesticides, including aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, and metalaxyl, on the earthworm *Aporrectodea caliginosa*, and observed a consistent decrease in growth rate and regenerative ability across all treatments. Similarly, Mosleh et al. [67, 79] also documented that endosulfan and aldicarb exposure led to significant

reduction in growth and suggested growth rate as a reliable biomarker for contamination in *Lumbricus terrestris*. Zhou et al. [80] reported that exposure to chlorpyrifos at 5 mg/kg concentration for eight weeks adversely affected growth and regeneration in *Eisenia fetida*, causing cellular degeneration in epidermal and muscle tissues. Moreover, Yasmin et al. [76] observed that juvenile earthworms are more sensitive to pesticide exposure than adults, suggesting that early developmental stages possess higher metabolic activity but lower detoxification capacity.

At the molecular level, pesticide exposure can induce oxidative stress through excessive generation of ROS, disrupt mitochondrial function, and alter the expression of key regeneration-associated genes such as Sox2, Oct4, TCTP, and Wnt3a. These effects lead to reduced cell proliferation, apoptosis dysregulation, and impaired blastema formation. Organophosphate pesticides, in particular, inhibit acetylcholinesterase, causing neurotoxicity that affects the neuroepithelial coordination necessary for anterior segment regeneration [40]. Persistent chemical stress may also trigger epigenetic modifications that reduce the activity of pluripotency networks, ultimately leading to regeneration failure.

Collectively, these studies establish that chemical pollutants especially pesticides pose a major threat to earthworm regeneration, making earthworm bioassays valuable tools in ecotoxicology and soil health monitoring. Table 5 summarizes the influence of various chemical agents, including pollutants and toxins, on the regenerative capacity of earthworms, highlighting their molecular and physiological impacts.

Table 5. Chemical factors affecting earthworm regeneration.

Factors	Earthworm species	Observed effect on regeneration and growth	Reference
Acetochlor (Herbicide)	*E. fetida*	Growth and regeneration suppressed; proposed as sensitive biomarker for toxicity.	[74]
Copper Oxychloride (Fungicide)	*E. fetida*	Reduced growth and reproductive rate; interferes with metabolic enzyme activity.	[75]
Carbendazim, Glyphosate, Dimethoate	*E. fetida*	Significant dose-dependent reduction in growth and regenerative success; coelomic fluid imbalance.	[76]
Parathion (Organophosphate)	*E. andrei*	Inhibits growth and cellular turnover; affects acetylcholinesterase activity and neuro-regeneration.	[77]
Chlorpyrifos and Diazinon	*E. fetida*	Marked suppression of motility and blastema development; degeneration of epidermal and muscle tissues.	[78, 80]
Aldicarb, Cypermethrin, Profenofos, Chlorfluazuron, Atrazine, Metalaxyl	*A. caliginosa*	General reduction in growth rate and regenerative ability across all treatments.	[67]
Endosulfan and Aldicarb	*L. terrestris*	Growth rate reduction; oxidative stress and apoptosis; identified as biomarkers for contamination.	[79]
Heavy Metals (Cd, Pb, Cu, Zn)	*L. terrestris*, *E. fetida*	mpair mitochondrial respiration and DNA repair; reduce coelomocyte viability and regeneration rate.	[29, 46]
Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)	*E. fetida*	Cause DNA damage and inhibit expression of pluripotency genes (*Sox2*, *Oct4*, *Nanog*).	[46]

Factors	Earthworm species	Observed effect on regeneration and growth	Reference
Retinoic Acid (Toxin)	*E. fetida*	Downregulates *Sox2* gene expression, leading to incomplete blastema formation and impaired anterior regeneration.	[52]

DISCUSSIONS

Regeneration in earthworms is not merely a biological phenomenon, but also serves as an adaptive strategy that ensures their population continuity and maintenance. This, in turn, directly sustains soil ecosystem dynamics and maintains terrestrial productivity. The remarkable regenerative ability of earthworms inevitably ensures their bioturbation functions, particularly when tillage practices, predation, or soil compaction produces physical damage to these animals. In this context, the regenerative ability of earthworms could also be reframed as their ecological trait, which is sensitive to anthropogenic stressors operating at landscape scales. The intricate interrelationship from molecular aspects of regeneration to its impact at an ecological scale is perhaps an underrated aspect of earthworm biology.

The present review encompasses both the mechanistic molecular mechanisms as well as the ecotoxicological aspects of earthworm regeneration. The ecotoxicological perspectives include the earthworms as a sensitive biomarker or bioindicator of soil contamination through the assessment of impact of contaminants on regeneration endpoints, such as blastema formation, regeneration time or rate of segment restoration and other parameters, particularly at sub-lethal concentrations. On the other hand, for the regenerative biologists, the earthworm can be used as a model organism to study the molecular consequences of challenging some specific pathways, which can be traced at the organism level in real time. Moreover, the anterior-posterior regeneration gradient in earthworms plays a significant role in population-level resilience modelling, particularly in agricultural fields, were tillage practices fragment earthworm populations regularly. The probability of anterior versus posterior injury in earthworms defines individual survival, population recovery and the restoration of their bioturbation functions.

The present review has included the studies in earthworm regeneration beyond organismal observations, supporting the extension of implications of regeneration directly to ecosystem resilience and environmental monitoring. Impaired regenerative capacity reduces earthworm survival and population density, thereby weakening soil bioturbation, nutrient cycling, and microbial symbioses. For example, pesticide-induced inhibition of blastema formation compromises individual recovery and diminishes soil aeration and fertility, linking molecular disruption to ecosystem-level decline. Similarly, anterior regeneration failures restrict sensory and locomotive functions, reducing the worms' ability to colonize and stabilize soil environments. By explicitly connecting regeneration outcomes to soil health and ecosystem services, this review situates annelid regeneration within the broader framework of ecotoxicology and environmental sustainability. Unlike earlier accounts that emphasized molecular pathways in isolation, the present synthesis integrates intrinsic biological determinants with extrinsic environmental stressors, thereby advancing regeneration research as both a biological process and a measurable ecological endpoint. This dual emphasis ensures that earthworm regeneration is recognized not only as a model for developmental biology but also as a critical bioindicator of environmental stress.

Knowledge gaps and future perspectives

Despite the scientific studies to date, several aspects of regeneration in earthworms lack comprehensive and detailed understanding as a complete biological process. This particular area of study has limited genomic or transcriptomic data, incomplete understanding of clitellum-mediated signalling and origin of blastemal cells, lack of epigenetic regulatory mechanisms and inadequate interpretation of ecological perspectives. Future research in this field might include the studies on epigenetic regulation, functional genomics and sequencing approaches, comparative studies across different species and regulatory factors and its applications in ecology and biomedicine.

CONCLUSIONS

Earthworms represent an important and unique model for understanding the mechanisms involved in regeneration, in combination with cellular plasticity and ecological importance. The present review outlines the cellular, physiological, and molecular phenomena involved in the sequential phases of wound healing, blastema formation, differentiation, re-segmentation, and growth, along with the regulatory factors and genes. The clitellum serves key roles in regenerative processes, such as stem cell reservoirs, and releasing many essential pluripotency factors of regeneration, such as TCTP, VEGF, and YAP1, governing its crucial role in regenerative processes. The present review also encompasses the species-specific variations among the different earthworm species, as well as the factors influencing the regenerative mechanisms. Several factors, such as environmental contaminants, including pesticides, may disrupt the regenerative processes by inducing oxidative stress, altering the gene expressions, and inducing cellular apoptosis. The key highlights of the present review emphasize earthworm regeneration with dual relevance: as a potent bioindicator for ecotoxicological studies, as well as a model for the study of molecular and developmental biology.

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Conflict of interest

The authors declare no conflict of interest.

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