Review

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*Review*

# Recent Light on the Developmental Perspectives of Regeneration in Annelids

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**Abstract:** Annelids are well-known for their ability to regenerate. The morphological development in terms of regeneration physiology, involves several cellular factors which play crucial roles in the developmental phenomena. The physiological process of regeneration involves critical biomechanical processes which is effectively regulated by the cellular factors. In today's biological research, the fundamental analysis of regeneration is necessary for the scientific assessment of cellular-biomechanical advancements, which eventually helps to develop adequate insight in the biomedical research in regenerative sciences. In this review we have elucidated the recent research on explaining the developmental physiology of regeneration in annelids.

**Keywords:** Regeneration; Annelids; Morphological development

## Introduction

The development of the ability to regenerate in multicellular organisms is one of biology's most intricate and fascinating issues. It is of concern, how could self-healing, an apparently beneficial characteristic, be continually diminished by evolution. The idea of regeneration's nature and origins, as well as its relationship to asexual reproduction, embryonic development, and tissue homeostasis mechanisms are elucidated. A range of traditional and contemporary theories are presented to explain various patterns in the evolution of regenerative ability, which is not always advantageous for the individual and especially not for the species. The growing limitation of differentiation flexibility and the development of signaling pathways, together with concurrent advancements in adaptive immunity, are the mechanisms behind these changes. In addition, cases of phylogenetically improved regeneration ability are examined, together with a description of the molecular processes behind the augmentation and the relevant evolutionary rationale [Elchaninov et al., 2021].

The principles behind effective function restoration and regeneration have long captivated researchers. The ability to start a developmental process that results in the partial or whole replacement of a damaged component differs greatly throughout animal taxa, even though most animals exhibit the ability to heal wounds. Within a same species, variation can also arise depending on the kind and location of the wound as well as the individual's age or developmental stage. Studies comparing the cellular and molecular alterations that take place before, during, and after wound healing may reveal shared genomic pathways in animals with varying capacities for regeneration. These discoveries have the potential to transform research in the field of regenerative medicine [Acosta et al., 2021].

Despite the common belief that annelids cleave, recent research has demonstrated that they can regulate the development of germline cells and other aspects of the embryo. However, the post-larval development of annelids exhibits the greatest diversity of regulatory systems. These animals exhibit different manifestations of what is likely an ancestral capacity to rejuvenate. Certain animals cannot grow back missing parts. But most animals can regenerate damaged posterior body parts, many can reconstruct lost head segments and features, and some can even build a whole body from scratch based on just one or two segments. A population of undifferentiated cells derived from the division of dedifferentiated and/or stem cells forms most of the regenerated structures. Furthermore, the process of regeneration frequently entails the modification of body fragments that have survived; as a result, it may be linked to both local and overall organism responses. The regeneration of the neurological, digestive, and integumentary systems, as well as the role of stem and undifferentiated cells in the formation of blastema and the replacement of missing gonads, are given consideration. The collection and examination of current research on the variety of cellular sources and mechanisms underlying annelid regeneration may provide insights into the processes that lead to the loss (limitation) of an ancestral characteristic of an animal as well as the most evolutionarily conserved programs for preserving regeneration capacity [Nikanorova et al., 2020].

Why animals differ in their capacity for regeneration is still one of biology's most fascinating mysteries. Within the vast and varied phylum of annelids, several individuals possess the ability to regenerate extensively, including growing a new head or tail or regrowing the entire body from a few parts. Conversely, certain members of the two main clades of annelids exhibit very little tissue regeneration and are not able to regenerate segments at all. Comprehending the many cellular and molecular underpinnings of regeneration in annelids will facilitate the resolution of significant inquiries regarding the function of stem/dedifferentiated cells and "molecular morphallaxis" in annelid regeneration, in addition to the evolution of regeneration broadly speaking [Kostyuchenko and Kozin, 2021].

The function of morphallactic and epimorphic events in regeneration and asexual reproduction in annelids is the topic of concern for developmental biologists. It was demonstrated that from the very beginning of regeneration processes, tissue rearrangement and molecular alterations already take place. These findings suggest that morphallaxis is necessary for the de novo synthesis of missing pieces in new zooids during asexual reproduction as well as for all successive stages of complete epimorphic regeneration, including initiation and completion [Kostyuchenko and Kozin, 2020].

The most rudimentary bilateral triploblastic creatures are represented by planarians. The regeneration of their cephalic ganglion following total excision serves as an example of how most planarian species demonstrate mechanisms for whole-body regeneration. Planarians have served as model organisms in regenerative research for more than 240 years due to their strong ability to regenerate their whole body. The processes behind planarian regeneration have gradually become clearer thanks to advancements in research instruments and methodologies. Regeneration is known to be critically dependent on accurate cell-to-cell communication. Recent decades have seen the molecular processes behind this kind of communication come to light. Notably, it has been determined that during planarian homeostasis and regeneration, stem cells-neoblasts-are the source of all new cells. During homeostasis and regeneration, the interactions between neoblasts and somatic cells influence the characteristics and sizes of different tissues [Chen and Lei., 2023].

## Regeneration in Annelids

In animals, regeneration, the capacity to replace lost or damaged body parts, is a common yet incredibly complicated and varied phenomena. Animal regeneration has interested scientists for decades, yet key issues regarding its evolutionary history and molecular foundation remain largely unresolved. The regeneration of the marine annelid *Platynereis dumerilii*, a model organism for comparative developmental biology that, like many annelids, demonstrates significant regenerative capacities. After losing a portion of their body, *P. dumerilii* worms may regrow that portion as well as form a growth zone rich in stem cells, which enables the worms to replace the lost segments with new ones. The posterior regeneration is a quick process that proceeds through distinct phases with well-repeated course and schedule. One day following amputation, wound healing is accomplished, and the following day, a regeneration blastema begins. As early as three days following amputation, a functioning posterior growth zone is restored, and some tissue specification has already taken place. Worm size has just a little effect on when regeneration occurs. Except for cases in which the amputation planes are quite near to the throat, comparable regenerating capacities are seen for amputations carried out at various points along the worm's antero-posterior axis. Regenerative capacities endure many amputations without significant changes to the process. Vigorous cell proliferation takes place during regeneration and that proper regeneration requires cell divisions.

Finally, pulse and chase studies using 5-ethynyl-2'-deoxyuridine (EdU) indicate that most blastemal cells originate from the area that immediately borders the amputation plane. Future mechanistic and comparative investigations of regeneration in *P. dumerilii* will have a solid basis to the thorough characterization of posterior body regeneration provided in this research [Planques et al., 2019].

Biologists find the earthworm especially intriguing due of its powerful ability to regenerate. Many facets of its regeneration in nature are still unknown, though. Single-cell RNA-sequencing, large-scale transcriptome, and chromosome-level genomic data collected throughout the regeneration process of earthworms (*Eisenia andrei*). The increase of gene families and LINE2 transposable elements that are functionally linked to regeneration (such as EGFR, the epidermal growth factor receptor), especially for genes that show differential expression throughout the regeneration process in earthworms. Transcriptional regulatory factors that may be essential for starting cell proliferation and differentiation during regeneration are identified by temporal gene expression trajectories. Moreover, transcriptional activation of early growth response genes associated with regeneration occurs in both planarians and earthworms. In the meanwhile, single-cell RNA sequencing sheds light on the biological mechanisms behind regeneration and reveals that stem cells make up most of the cells present during regeneration [Shao et al., 2020].

It has been demonstrated that the genetically determined, endogenously mediated segment regenerating capability of the model animal is overridden when excised *Eisenia andrei* earthworms are exposed to Terahertz (THz) radiation. Single-cycle THz pulses with 5 μJ energy, 0.30 THz mean frequency, 293 kV/cm peak electric field, and 1 kHz repetition rate stimulated both histogenesis and organogenesis, leading to a significantly higher number of regenerated segments. Cell proliferation was indicated by the high number of mitotic cells. The more rapid formation of new blood vessels and the central nervous system in THz-treated mice was the most obvious change. These findings imply possible medical uses for THz pulses and unequivocally show that they may effectively initiate biological processes [Abufadda et al., 2021].

Annelids include earthworms. They are essential to soil fertility and agriculture. The greatest organic manure for plant crops is vermicompost. One earthworm that works well for producing vermicompost is *Eudrilus eugeniae*. Because of the worm's fast growth rate, quick reproduction, tolerance to a broad variety of temperatures, and low care costs, it is also used to investigate developmental biology, molecular toxicity, and cell and molecular biology of regeneration [ Arumugaperumal et al., 2022].

Earthworms are well known among annelids for their extraordinary capacity to repair missing segments. The 120 segments that make up the adult earthworm *Eudrilus eugeniae* are separated into three categories: pre-clitellar, clitellar, and post-clitellar segments. The results of this study indicate that clitellum is essential to the species' effective regeneration. To determine which of the earthworm's three skin layers, the cellular layer supporting the production of blastemas and the species' regeneration. According to the histology data, the earthworm's ability to regenerate successfully depends on the longitudinal cell layer at the amputation site proliferating, which can only happen while the clitellum is intact. Additionally, to track the important genes that are differently expressed and the pathways and activities that are connected to them that regulate the clitellar tissue changes during the earthworm's anterior and posterior regeneration. Between the control clitellum and the clitellum of anteriorly regenerated earthworms, 4707 Differentially Expressed Genes (DEGs) were found, and between the control clitellum and the clitellum of posteriorly regenerated earthworms, 4343 DEGs were found. In the clitellar tissue during both anterior and posterior regeneration of the earthworm, the functional enrichment analysis confirmed that the genes regulating the muscle mass shape and structure were significantly downregulated and the genes related to response to starvation and anterior-posterior axis specification were significantly upregulated. The comparative transcriptome analysis and the RNA sequencing data of Clitellum were useful in understanding the intricate mechanism of earthworm regeneration [Paul et al., 2022].

The annelid *Capitella teleta* has been utilized more frequently as a research system in the past several decades to examine growth and regeneration. Its favourable traits include considerable regeneration capacities, a stereotypic cleavage programme of early development, the availability of a sequenced genome, the capacity to sustain a laboratory culture continuously, and well-established experimental and functional genomics approaches. Every research system has a distinct history, and each animal has pros and cons when used as a test subject. Developing tools and resources for a less researched species requires strategy, perseverance, and dedication, much like other scientific advances [Seaver et al., 2022].

One of the three bilaterian superclades is the Lophotrochozoa, which includes the segmented worms, often known as annelids. Because of their remarkable capacity for regeneration, annelids have long been used as models in research on regeneration. Moreover, the group shows variety in adult regeneration capacities, with certain species having the capacity to replace one or both anterior and posterior segments. Complex organ systems, such as the centralized nervous system, stomach, muscles, nephridia, and gonads, can regenerate successfully. Because of its strong posterior regeneration, *Capitella teleta* is well-suited to examine the molecular and cellular mechanisms governing the regeneration response. *C. teleta*'s extremely stereotypical developmental program offers chances to research adult regeneration and produce reliable comparisons between development and regeneration [Seaver and Jong, 2021].

After *C. teleta* developed the ability to regenerate, we evaluated the degree of regeneration in larvae. The anterior and posterior wound sites displayed the stem cell marker vasa. However, only in posterior amputations was fresh tissue development seen. At the posterior wound locations, ventral nerve cord neurons were also visible. While the lack of elav expression suggested an incapacity to advance to neuronal differentiation, de novo ash expression in the ectoderm of anterior wound sites showed neuronal cell specificity. Cilia and eyes reformed in uncommon cases. In preexisting tissues, both amputations led to increased expression of the myogenesis gene MyoD. The findings show that severed larvae finish early phases of regeneration but not late ones, suggesting that *C. teleta* gradually acquires the capacity to regenerate [Boyd and Seaver, 2023].

Because echiuran adults lack metameric body segmentation, in contrast to most other annelids, Echiura is one of the most fascinating significant subgroups within the phylum Annelida. Soft sediments form U-shaped tunnels that are home to *Urechis unicinctus*. The molecular processes that underlie *U. unicinctus* development are poorly understood. Here, we used immunohistochemistry to examine the nervous system and F-actin labeling to examine the juvenile *U. unicinctus* developmental pathway from zygote to adult. We discovered that muscles for eating were formed initially and that muscle fibers started to develop in the trochophore phase using F-actin labeling. Later, during the segmentation larval stage, a ventromedian muscle was developed for the construction of a ventral nerve cord and a transversal muscle in the shape of a ring with segment formation in an anterior-posterior orientation. The entire body then began to develop many muscle fibers, which combined to form the larva's worm-like shape. The genes for smooth and striated muscle were co-expressed in the same area throughout embryonic development. The mature body wall muscles, however, had distinct gene expression for every muscle layer. The knowledge of muscle differentiation in Echiura will be based on the findings of this study [Han et al., 2020].

Polynoid scaleworms are recognized to be able to regenerate a variety of appendages and the posterior regions of the trunk, although this behavior is underappreciated. In polynoids and other annelids, autotomy requires the exterior cuticle, a collagenous structural substance, to break. The purpose of this study was to use light and electron microscopy to shed light on the mechanism by which the cuticle is fractured during trunk autotomy in *Harmothoe impar*. The cuticle breaks at a pre-formed breakage zone during autotomy, passing through a thickening of the cuticula that is made up of an external ridge and an interior apodeme. Cuticular fracture is thought to be caused by the contraction of certain longitudinal muscle fiber groups, which concentrates tensile tension at the base of the breakage zone. The external ridge, which prevents stress concentration at the breakage zone's exterior edge, and the breakage zone's undulating profile, which increases its resistance to shearing forces, are two morphological features that lessen the chance of accidental fracture of the zone [Wilkie et al., 2020].

Since the stimulation that initiates asexual reproduction or fission appears to be the sole distinction between the two, regeneration, the capacity to restore lost body parts, is a common occurrence in the animal kingdom. While most of these developmental processes have been described, little is known about the molecular machinery and genetic pathways that underlie these activities. Annelids, namely the oligochaete *Pristina leidyi*, offer a useful model organism for studying these processes since they can reproduce asexually in the lab by fission and exhibit a variety of regeneration strategies. 291 genes were identified to be elevated during anterior regeneration, including numerous genes linked to regeneration. However, 130 genes were revealed to be elevated during asexual reproduction, and surprisingly, many of these genes were connected to the formation of the germline during sexual reproduction. Important distinctions were also seen between asexual reproduction and anterior regeneration, with the latter exhibiting a gene expression profile more akin to that of control subjects. Nevertheless, we discovered 35 genes, many of which were connected to stem cells, cell proliferation, and pluripotency, that were increased in both circumstances [Olmo et al., 2022].

Although the process of regeneration has been extensively studied, the underlying molecular mechanism is still unknown, particularly in the early stages (wound healing and blastema production). This study examined the early regeneration using the recently discovered *Ophryotrocha xiamen*. The morphological alterations that occurred during the regeneration process (0–3 days after amputation) and the molecular changes that occurred during the early stage of regeneration (1 day after amputation) were examined. In just one day, the wound healed, and the following day, a blastema developed. The primary functions of 243 DEGs were signal transduction and metabolism. *O. xiamen* was shown to have regeneration-related genes, which may aid in deciphering the roles of genes implicated in regeneration processes. Hox5 and Lox2 were discovered to be missing in early regeneration and throughout ordinary growth, and we were able to identify eight distinct Hox gene segments based on their common pattern [Chen et al., 2022].

The transcriptome analysis of *Ophryotrocha xiamen*, we have discovered a regeneration-related gene called Oxfibrillin. This model proves to be a useful tool for investigating the processes of regeneration. Large glycoproteins called fibrillins combine to create microfibrils and control the transmission of growth hormones or other substances. Here, we were able to get Oxfibrillin's 31,274 bp genomic DNA sequences. 1927 amino acids containing a VWD domain, EGF/cb-EGF domains, a TR domain, and a transmembrane domain were encoded by the 5784 bp coding sequence. Oxfibrillin's homology scores to mammalian fibrillin were low, placing it in the subgroup of invertebrates. Oxfibrillin genes were consistently elevated throughout the early regeneration phase and subsequently remained stable until the development of the whole tail, according to gene expression study, suggesting that it may be a crucial element influencing the posterior regeneration process [Chen et al., 2023].

Among annelids, epimorphic regeneration of lost body segments is a common occurrence. Nevertheless, the cell sources' molecular inducers for this reparative morphogenesis remain unknown. Fibroblast Growth Factor (FGF) signaling in *Alitta virens* posterior regeneration plays a crucial role. An annelid recovering from an amputation exhibiting early activation of FGF ligands and receptor expression. The expression patterns show that FGFs, whose activity occurs before the start of cell proliferation, can activate the whole regenerative bud. The proliferation assay after inhibitor treatments supports the important need of FGF signaling, particularly in the early phases, and shows that FGFs are necessary for the induction of blastemal cells. The FGF-positive wound epithelium, the ventral nerve cord, and some mesodermal cells surrounding the gut may be the inciting tissues, according to the results, which indicate that the FGF signaling pathway is a major participant in the regeneration response. This process is like the reparative regeneration of vertebrate appendages, which suggests that all bilaterians may have evolved a similar reaction to damage [Shalaeva et al., 2021].

Annelids are exceptional postembryonic developing organisms. The Segment Addition Zone (SAZ), which is situated in front of the pygidium, allows most annelids to expand by adding segments during their whole lives. They also have a remarkable capacity for body regeneration. Many annelids can renew their posterior bodies, according to experimental data and field observations, whereas anterior regeneration is frequently restricted or nonexistent. While anterior regeneration differs throughout species, *Syllidae*, for example, often have significant posterior regeneration capacities. While some syllids can regenerate the whole anterior body following bisection, others can only repair a portion of the front end. 4771 genes in *S. hystrix* (limited anterior regeneration) and 1997 genes in *S. gracilis* (full anterior regeneration) were among the many genes with differential expression that were discovered. Comparative transcriptome study for both species revealed that whereas anterior regeneration was marked by multiple gene up-regulation, posterior regeneration and normal growth exhibited very similar gene expression patterns. Among the up-regulated genes, we found putative homologs of regeneration-related genes linked to the development of the nervous system, the establishment of the body axis, cellular proliferation, and stem-cellness, such as glutamine synthetase, elav, slit, Hox genes, β-catenin, and PL10 (in *S. gracilis*) and rup and JNK (in *S. hystrix*). In the syllids under investigation, there are no discernible variations in gene expression between posterior regeneration and ordinary development. Nonetheless, in both species, anterior regeneration is linked to a pronounced alteration in gene expression. Differential expression of several genes linked to regeneration was found by our comparative transcriptome study, indicating that syllids and other annelids and invertebrates may have some similarities in their regenerative processes [Ribeiro et al., 2021].

Methylation of cytosines in DNA, or 5mC methylation, is a significant epigenetic alteration that controls gene expression and forms the framework for many processes controlling various facets of vertebrate embryonic development and cell reprogramming. In mammals, transcriptional inhibition is associated with 5 mC methylation of promoter regions. The nucleosome remodelling and deacetylase complex (NuRD complex), which connects DNA methylation and histone alterations, is a significant player in the control of transcription by 5 mC methylation. Less is understood, meanwhile, regarding the role of regulatory processes involving 5mC methylation in non-vertebrate species. Here, we examine 5mC methylation in the marine annelid worm Platynereis dumerilii, a model for developmental biology and evolution that can regenerate the posterior region of its body after amputation. *P. dumerilii* has a high degree of DNA methylation like that of mammalian somatic cells using both in silico and experimental methods. In *P. dumerilii*, 5mC methylation is dynamic during the animal's life cycle and significantly drops as the larval to post-larval stages approach. We show that *P. dumerilii* possesses a complete repertoire of mostly single-copy genes encoding the components of the NuRD complex or the machinery linked to 5mC methylation, and that this repertoire is like that suggested for the last common ancestor of bilaterians. During the development and regeneration of *P. dumerilii*, these genes express themselves dynamically. *P. dumerilii* larval development and regeneration are hampered by treatment with the DNA hypomethylating drug Decitabine, which also affects post-regenerative growth in the long run. The use of a chemical DNA methylation inhibitor, analysis of machinery gene expression throughout development and regeneration, and DNA methylation levels all point to the role of 5mC methylation in *P. dumerilii* development and regeneration. Additionally, we give evidence that suggests *P. dumerilii* is a suitable model for investigating the biological activities and processes of DNA methylation in non-vertebrate bilaterians, as well as for offering fresh insights into the development of this important epigenetic change in bilaterian species [Planques et al., 2021].

## Discussion

All metazoans exhibit the common phenomena of RNA editing. The chemical conversion of adenosine to inosine (A-to-I), which is mediated by adenosine deaminases acting on tRNA (ADAT), is one of the frequent RNA editing events. The ADAT1 transcript was discovered to localize mostly to the central nervous system, which includes the brain and ventral nerve cord, during the development of the brain in *Drosophila. melanogaster*. There isn't much information on ADAT and tRNA editing in annelids, especially terrestrial earthworms, despite the identification of an earthworm adenosine deaminases acting on mRNA (ADAR) and research into its potential role in earthworm regeneration [Yoon et al., 2021].

Within the Lophotrochozoa, segmented worms, or annelids, constitute a prominent group. They belong to a sizable and significant phylum in terms of ecology. They exhibit a great range of bodily types coexisting with different approaches to living. The two primary clades of annelids are Errantia and Sedentaria. For the past 20 years, the Errantia's *Platynereis dumerilii*, an emerging model species, has led the way in developmental biology, evolution, and ecology. This chapter covers the following topics: (i) the fundamental biology of this model species, including its dramatic life cycle that is synchronised with the phases of the moon; (ii) its anatomy, which shows dramatic alterations during sexual maturity; and (iii) the key developmental milestones [Schenkelaars and Gazave et al., 2021].

In sexually reproducing organisms, germ cells, reproductive cells and their progenitors, give birth to the following generation. Sterility is frequently the result of losing or removing germ cells from well-established study species such fruit flies, nematodes, frogs, and mice. The belief that germ cells are set aside during embryogenesis and cannot be replaced by somatic cells—the germline, soma barrier, was strengthened by the failure of these creatures to regenerate germ cells. On the other hand, a wide variety of creatures, such as tunicates, hydrozoans, planaria, sea stars, sea urchins, segmented worms (annelids), and sea stars, are capable of regeneration of germ cells. Annelids from various phylogenies, developmental phases, and reproductive techniques are a few examples. As part of their asexual reproduction, grafting, and regeneration processes, adult annelids replenish germ cells. After the embryos' germ cell progenitors are ablated, annelids are also capable of recovering germ cells. With the use of modern genetic lineage tracking and bioinformatics methods, together with several genetically enabled annelid models, we may soon be able to provide answers to the major mysteries that have baffled people for more than a century [Özpolat, 2023].

The study of anatomical abnormalities has been more important for understanding development and its evolution in recent decades. However, this viewpoint has never been used to study most animal species. Anteroposterior axis bifurcation, or having two or more heads and/or tails, is one of the most prevalent and notable abnormalities among annelids. Since their original description in the 18th century, bifurcated annelids have been occasionally recorded. Nevertheless, a succinct but thorough explanation of this occurrence is absent, as these animals have seldom been regarded as much more than oddities, anomalies, or monsters. We outline the several anatomies that are shown in the reports of annelid bifurcation that have been published over the previous 275 years in this study. According to our survey, bifurcation is a common occurrence across the annelid tree. Additionally, it demonstrates how bifurcations may be categorized into several categories based on developmental origin (embryonic vs postembryonic, the latter happening in connection to regeneration, reproduction, or growth) or architecture (lateral versus dorsoventral). Three distinct forms of bifurcation are present in embryos: *duplicitas cruciata* embryos (which have anterior and posterior bifurcation with a 90° rotation); conjoined twins (found in clitellates); and Janus embryos (found in two posterior ends with a single head that display duplicated features). We demonstrate that in adults, dorsoventral bifurcation cannot produce well-integrated phenotypes because it necessitates the discontinuity of at least some internal organs. However, lateral bifurcation may. The Ribbon Clade is a group of syllid annelids in which some species reproduce by collateral and consecutive gemmiparity (including dorsoventral bifurcation), while other species develop by branching horizontally. This illustrates the significance of this feature. Most documented occurrences of bifurcation are either inadvertently formed or the result of accidental discoveries in the wild; nevertheless, experimental research that induce bifurcation in both adults and embryos are also examined. These experimental experiments demonstrate how zygote bifurcation may occur in embryos when there is mechanical or chemical disturbance. In adults, the induction of bifurcation appears to be influenced by the digestive tract and the ventral neural system. Considering the reviewed facts, the long-neglected research of annelid developmental abnormalities ought to be brought into the expanding area of annelid EvoDevo and investigated using contemporary methods and viewpoints [Ponz-Segrelles et al., 2022].

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