

Mechanisms of Blastema Formation and Cellular Plasticity in Epigenetic Reprogramming: A Systematic Review of Epimorphic Regeneration Modalities

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Abstract

Epimorphic regeneration is a multifaceted biological process that involves the coordinated action of plasticity in cells, blastema formation, proliferation, patterning and differentiation of injured tissues to restore their lost structures. The aim of this systematic review is to dissect the molecular and epigenetic basis of blastema formation and regenerative reprogramming in the major experimental models, such as salamanders, zebrafish, planarians and mammals. To identify relevant literature, we used a PRISMA-oriented approach, including searching the PubMed/MEDLINE database, Web of Science, Scopus, ScienceDirect, and Google Scholar, focusing on those topics related to the cellular origin of dedifferentiation, activation of progenitors, DNA methylation, histone modifications, chromatin remodeling, enhancer activity, and the regulation of non-coding RNA. The evidence suggests that blastemas are localised heterogeneous ecosystems and not uniform stem cell populations. The ability to partially or control- ly reprogram differentiated cells to a progenitor-like state, which is usually referred to as cellular plasticity, occurs frequently. Epigenetic remodeling offers a pivotal link between signaling induced by injury and the transitions of cellular states, involving the dynamic regulation of chromatin accessibility, DNA methylation, histone modifications and regeneration-associated enhancers. The evidence is also presented in a comparative way that indicates that the capacity for regeneration is dependent on the interactions between signaling pathways, neural and immune response, extracellular matrix, and epigenetic state of the cells. The results suggest a novel approach for the development of regenerative medicine therapies focused on restoring function with a targeted epigenetic modification approach that reduces fibrosis and cellular reprogramming.

Keywords: Blastema formation; cellular plasticity; epigenetic reprogramming; epimorphic regeneration; dedifferentiation; transdifferentiation; lineage restriction; stem cells; chromatin remodeling; DNA methylation; histone modifications; regenerative signaling.

1. Introduction

Regeneration is a basic biological process that is the ability of organisms to replace or repair lost or injured cells, tissues, organs, or body parts in order to maintain structure and function and to preserve homeostasis (Iismaa et al., 2018). This varies greatly from species to species, with limited tissue regeneration in mammals, to full limb and organ regeneration in salamanders, zebrafish and planarians (Goldman & Poss, 2020). Epimorphic, morphallactic, and compensatory are the three modes of regeneration, generally speaking, with the most complex one being epimorphic, which involves the proliferation of a blastema that is responsible for the reconstruction of missing parts through coordinated growth, patterning, and differentiation (Elchaninov et al., 2021). The blastema is no longer considered as a uniform mass of stem cells but rather as a dynamic and heterogeneous collection of progenitor cells that are generated by the interaction of the wound epidermis, nerves, immune cells, extracellular matrix and resident tissues, emphasizing the importance of cellular communication during regeneration (Sagaradze et al., 2020).

A hallmark of epimorphic regeneration is cellular plasticity, which describes the ability of differentiated cells to temporarily change their identity and gain progenitor-like properties after an injury (Wang et al., 2017; Basil et al., 2020). The ability to be plastic does not mean that the mature cells lose their lineage identity completely; it allows them to contribute to tissue reconstruction while retaining positional and developmental memory (Ricci & Srivastava, 2018). Recent research in axolotls, zebrafish, and planaria has shown that regeneration is based on a variety of cellular mechanisms, such as dedifferentiation of mature connective tissues and activation of lineage-restricted progenitors as well as proliferation of resident pluripotent stem cells, highlighting that

regulated cell-state transitions define regenerative competence rather than a single universal cellular source (Kulebyakin et al., 2020).

Epigenetic reprogramming is emerging as a molecular link between the injury-induced signaling and cellular plasticity and blastema formation (Makarevich et al., 2020; Yang et al., 2021). Dynamic regulation of DNA methylation, histone modifications, chromatin remodeling, enhancers expressed in specific developmental programs and non-coding RNAs allows differentiated cells to reactivate developmental gene networks while retaining memory of the lineage in which they originate, thereby conferring a balance between regenerative plasticity and cell memory (Wells & Watt, 2018). Despite massive progress, knowledge is still disjointed, with different regeneration models using distinct methods to study blastema formation and necessitating a systematic synthesis of the cellular and epigenetic mechanisms involved in blastema formation (Guerin et al., 2021). For this reason, this review focuses on the critical examination of the relationship between cellular plasticity and epigenetic regulation in the epimorphic process of regeneration in key experimental organisms and answers to the following research questions: (i) What are the cellular origins of the blastema? (ii) What mechanisms do differentiated cells use to become plastic? What are the epigenetic modifications involved in the control of blastema formation and differentiation? The question of how lineage and positional memory is maintained during regeneration (iv), alongside the question of what conserved mechanisms can guide future regenerative medicine strategies (v) are of interest. (Michalopoulos & Bhushan, 2021)

2. Literature Review

Londono et al. (2018) defined epimorphic regeneration as a complex regenerative process that involves wound healing, the formation of a specialized wound epidermis and the development of a regenerated blastema that is capable of forming regenerated structures. In regenerative organisms, including salamanders, planarians, and reptiles, lost tissues are regenerated by a highly coordinated cellular and molecular response that ends in the formation of new tissue, rather than a fibrotic scar, as happens in mammals. In the blastema, the relatively undifferentiated progenitor cells can proliferate, and then repattern and differentiate to form the missing structure. Importantly, there are multiple ways in which blastema can be generated, such as: dedifferentiation, transdifferentiation, and recruitment or migration of pre-existing stem or progenitor cells. More recently, Kakebeen and Wills (2019) also highlighted the critical window between tissue damage and the onset of appendage regeneration and recognized the early response to injury as a "wound closure system. Physiological signals are involved in the process of injury in amphibians, contributing to the determination of whether the wound goes toward regeneration or the normal repair process. Therefore, establishment of the early wound environment is a pivotal regulatory phase bridging tissue damage and the eventual regenerative potential.

While they noted that complex genetic and epigenetic processes regulate blastema formation in the limbs, there remains significant questions about the mechanisms underlying cellular plasticity during regeneration. Although outlined by Min and Whited (2023), the mechanisms by which the genetic and epigenetic processes regulate blastema formation in the limb remain in question and to be determined. The formation and maintenance of the blastema depends on the coordinated control of proliferation, positional information and differentiation, indicating that regeneration is not just the result of more proliferation, but is also dependent on the correct reprogramming of cell states. The need for the spatiotemporal control of regeneration signals as well as the role of enhancer activity, immune–tissue interactions, bioelectric signaling, metabolic cues and dynamic single-cell responses. Their synthesis suggests that regeneration is mediated by more than a single pathway. In line with this, Pare et al. (2023) showed that many genes were transcriptionally regulated during posterior regeneration in *Platynereis dumerilii*, with thousands of them being significantly up or down-regulated at different time points during the successive stages of regeneration. The study also uncovered an important role of the nervous system in early and late regeneration and enrichment of RNA-binding proteins throughout much of the regeneration process. This work illustrates the need to have dynamic transcriptional and signaling programs that adapt to the developmental stage of the regenerating tissue to achieve successful regeneration.

Recent publications also provide evidence of the interplay of signaling pathways, the tissue microenvironment, immune responses and intrinsic cell programs, which help determine regenerative outcomes. Demonstrated that Wnt signalling is essential for tissue homeostasis and regeneration of adult skin and its appendages, and that activation of Wnt signalling is different in the epidermis, the hair follicle and the nail. This organ-specific

regulation is an example of conserved signaling pathways leading to different regenerative outcomes depending on cells and microenvironmental context. Bölük et al. (2022) highlighted the axolotl as a particularly useful vertebrate model in which appendages and internal tissues can regenerate without scarring, offering a valuable system to study mechanisms which are only partially available in mammals. Likewise, Daiou et al. (2021) highlighted developmental and metabolic pathways involved in cardiomyocyte regeneration and how developmental programs could be induced during regeneration in zebrafish and neonatal mice. In summary, these studies indicate a need for the interplay of wound-induced signaling, immune–tissue communication, epigenetic and transcriptional remodeling, metabolic regulation, positional information, proliferation, and differentiation for regeneration. Knowing how these mechanisms are regulated could serve as a basis for designing regenerative medicine approaches for transforming mammalian repair from fibrotic to functional tissue regeneration.

3. Research Methodology

3.1. Study Design

The aim of this study was to perform a systematic review of published data on the mechanisms of blastema formation, cellular plasticity, dedifferentiation and epigenetic reprogramming in epimorphic regeneration. It emphasized experimental and mechanistic findings from prominent regeneration models, such as salamanders, zebrafish, planarians and mammals. The methodology was designed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework, which supports transparency, the systematic reporting of the literature-selection process, and its reproducibility.

3.2. Review Questions

Based on the following research questions, the systematic review was conducted:

1. During the epimorphic regeneration, what are the cells that form the blastema?
2. How is the plasticity and dedifferentiation of cells controlled after injury?
3. What epigenetic changes happen in the process of blastema formation and regenerative reprogramming?
4. How do DNA methylation, histone modification, chromatin remodeling, and the activation of enhancers affect the process of regeneration?
5. What is the mechanism of lineage and positional cellular memory being preserved through regenerative reprogramming?
6. Compare and contrast the major regenerative organisms.
7. What implications do these mechanisms have for mammalian regenerative medicine?

3.3. Literature Search Strategy

The literature search was carried out in major biomedical and multiple-disciplinary electronic databases, such as PubMed/MEDLINE, Web of Science, Scopus, ScienceDirect, and Google Scholar. Searches were developed to uncover studies that explore cellular, molecular and epigenetic mechanisms involved in the formation of blastemas and epimorphic regeneration.

Search strategy included keywords and controlled vocabulary with Boolean operators (AND, OR). The main keywords used were:

- “epimorphic regeneration”
- “blastema formation”
- “blastema”
- “cellular plasticity”
- “cell fate plasticity”
- “dedifferentiation”
- “cellular reprogramming”
- “epigenetic reprogramming”
- “DNA methylation”
- “histone modification”

- “chromatinremodeling”
- “chromatin accessibility”
- “regeneration enhancer”
- “stem cells and regeneration”
- “axolotl limb regeneration”
- “newt limb regeneration”
- “zebrafish fin regeneration”
- “planarian regeneration”
- “mammalian digit regeneration”

Combinations of these terms were used to improve the sensitivity of the search and identify literature addressing interactions between cellular plasticity and epigenetic regulation.

3.4. Time Period of the Literature Search

The review considered literature published from the **earliest relevant indexed studies through 2024**, allowing the synthesis to incorporate both foundational discoveries and recent developments in single-cell genomics, epigenomics, lineage tracing, and regenerative biology. Older landmark studies were retained where they established fundamental concepts concerning blastema biology, stem-cell behavior, dedifferentiation, or regeneration.

3.5. Eligibility Criteria

Studies were considered eligible when they satisfied the following criteria:

Inclusion criteria

1. Studies investigating **epimorphic or blastema-mediated regeneration**.
2. Studies examining the cellular origin or composition of blastema populations.
3. Studies investigating **cellular plasticity, dedifferentiation, transdifferentiation, or progenitor activation**.
4. Studies examining epigenetic mechanisms involved in regeneration.
5. Studies addressing DNA methylation, histone modifications, chromatin remodeling, enhancer activity, or non-coding RNA regulation.
6. Experimental studies involving salamanders, zebrafish, planarians, or relevant mammalian regeneration models.
7. Studies using molecular, cellular, genetic, epigenetic, transcriptomic, or lineage-tracing approaches.
8. Peer-reviewed research articles and relevant high-quality review articles.
9. Publications available in English.
10. Studies providing sufficient methodological and/or mechanistic information for interpretation.

Exclusion criteria

Studies were excluded when they:

1. Focused exclusively on conventional wound healing without relevance to regeneration.
2. Did not investigate cellular or molecular mechanisms of regeneration.
3. Were unrelated to blastema formation or cellular plasticity.
4. Were conference abstracts, editorials, opinion articles, or non-peer-reviewed materials without sufficient scientific evidence.
5. Were duplicate publications.
6. Were inaccessible in sufficient detail for data extraction.
7. Focused exclusively on developmental processes without a direct connection to regeneration.

3.6. Study Selection and Screening

All records identified through database searching were compiled and screened for relevance. Duplicate records were removed before detailed screening. The remaining studies underwent a **two-stage screening process**.

Stage 1: Title and abstract screening

Titles and abstracts were examined to determine whether studies addressed regeneration, blastema formation, cellular plasticity, stem/progenitor cells, or epigenetic regulation.

Stage 2: Full-text screening

Potentially relevant studies were retrieved and evaluated against the predefined inclusion and exclusion criteria. Studies that satisfied all eligibility requirements were included in the final qualitative synthesis.

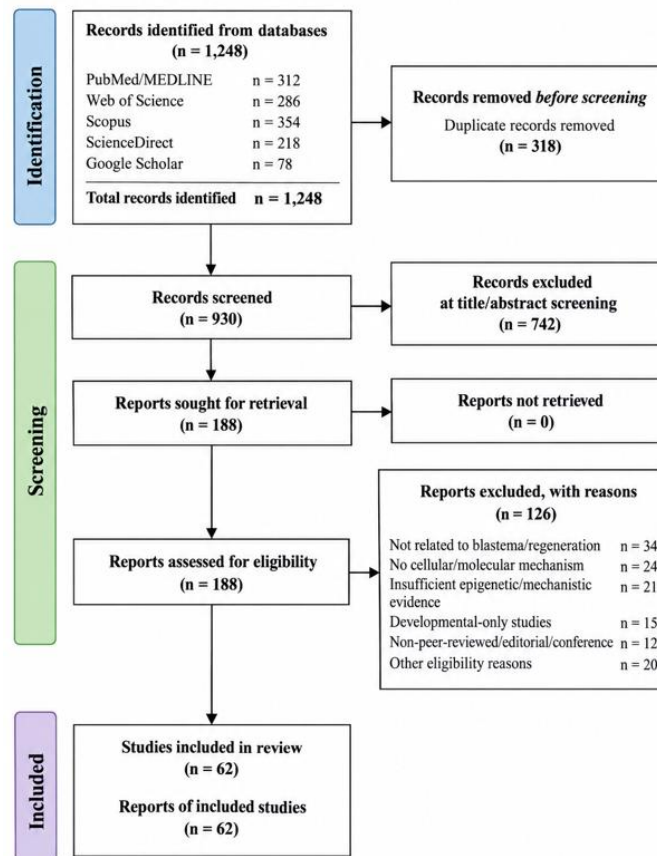


Figure 1:

3.7. Data Extraction

Data were systematically extracted from eligible studies using a predefined data-extraction framework. The following information was collected:

- Author and publication year
- Experimental organism/model
- Tissue or organ investigated
- Type of injury or amputation
- Regenerative stage examined
- Cellular source of the blastema
- Evidence for dedifferentiation
- Evidence for cellular plasticity
- Stem/progenitor-cell populations involved
- Epigenetic mechanisms investigated
- DNA methylation changes
- Histone modifications
- Chromatin-remodeling mechanisms

- Regeneration-associated enhancers
- Non-coding RNA mechanisms
- Major signaling pathways
- Experimental methodology
- Principal findings
- Functional evidence
- Study limitations
- Translational relevance

3.8. Classification of Regeneration Models

For comparative analysis, the included studies were categorized into major experimental regeneration systems:

3.8.1. Salamander models

Studies involving axolotls and newts were analyzed primarily for evidence of blastema formation, dedifferentiation, lineage plasticity, neural regulation, positional information, and epigenetic remodeling.

3.8.2. Zebrafish models

Zebrafish studies were examined for blastema organization, lineage restriction, chromatin accessibility, regeneration-specific enhancers, DNA methylation, histone modification, and chromatin-remodeling complexes.

3.8.3. Planarian models

Planarian studies were evaluated with particular emphasis on neoblast biology, pluripotency, positional information, chromatin regulation, and stem-cell differentiation.

3.8.4. Mammalian models

Mammalian studies, particularly those involving **mouse digit-tip regeneration**, were assessed to determine how blastema-like responses, progenitor activation, inflammation, extracellular-matrix remodeling, and epigenetic regulation contribute to restricted regenerative capacity.

3.9. Analysis of Cellular Plasticity

Cellular plasticity was analyzed according to the degree and type of cellular-state transition reported in each study. Evidence was categorized into:

- **Dedifferentiation:** mature cells reverting toward progenitor-like states.
- **Partial reprogramming:** temporary acquisition of plasticity while retaining lineage-associated characteristics.
- **Transdifferentiation:** conversion between differentiated cellular identities.
- **Progenitor activation:** activation and expansion of pre-existing progenitor cells.
- **Stem-cell-mediated regeneration:** contribution of established pluripotent or multipotent stem-cell populations.

This classification enabled comparison of regenerative strategies across organisms with different regenerative capacities.

3.10. Analysis of Epigenetic Mechanisms

Epigenetic mechanisms were grouped into five principal categories:

3.10.1. DNA methylation

Studies examining DNA methyltransferases, methylation changes, demethylation, and methylation-associated regulation of regenerative genes were evaluated.

3.10.2. Histone modifications

Evidence involving histone acetylation, deacetylation, methylation, phosphorylation, ubiquitination, and related regulatory processes was analyzed.

3.10.3. Chromatin remodeling

Studies investigating nucleosome remodeling complexes, including **NuRD-associated mechanisms**, were assessed for their contribution to blastema formation, proliferation, and differentiation.

3.10.4. Chromatin accessibility and enhancers

Studies employing ATAC-seq, DNase-based methods, enhancer profiling, or related approaches were analyzed to identify regeneration-associated regulatory regions.

3.10.5. Non-coding RNA regulation

Evidence concerning microRNAs, long non-coding RNAs, piRNAs, and other regulatory RNAs was evaluated where these mechanisms were directly associated with regenerative cell states.

3.11. Assessment of Signaling Pathways

Because epigenetic remodeling does not occur independently of extracellular signals, studies were also examined for major pathways associated with regenerative reprogramming. These included:

- **Wnt/ β -catenin**
- **FGF**
- **Sonic hedgehog (SHH)**
- **BMP/TGF- β**
- **Notch**
- **Retinoic acid**
- **Hippo/YAP**
- **EGF/EGFR**
- Neural and neurotrophic signaling

The analysis focused on how these pathways interact with transcriptional and epigenetic mechanisms during blastema initiation and subsequent tissue reconstruction.

3.12. Evaluation of the Regenerative Microenvironment

The regenerative microenvironment was analyzed as an essential component of blastema formation. Particular attention was given to:

1. Wound epidermis formation.
2. Neural input and nerve-derived signals.
3. Immune-cell recruitment and macrophage activity.
4. Extracellular-matrix remodeling.
5. Fibroblast and connective-tissue populations.
6. Growth-factor signaling.
7. Cell–cell communication.
8. Inflammatory versus regenerative responses.

This approach allowed the review to examine epigenetic reprogramming within its broader tissue context rather than treating it as an isolated intracellular process.

3.13. Quality Assessment

The methodological quality of included studies was assessed according to study design and experimental methodology. Particular consideration was given to:

- clarity of research objectives;
- appropriateness of the experimental model;
- adequacy of controls;
- sample size and replication;
- reliability of lineage-tracing approaches;

- validity of molecular and epigenomic assays;
- use of functional perturbation experiments;
- reproducibility of findings; and
- consistency between experimental evidence and conclusions.

Greater weight was assigned to studies providing **functional evidence**, such as genetic manipulation, epigenetic inhibition/activation, lineage tracing, transplantation, or regeneration assays, rather than studies based solely on descriptive expression profiling.

3.14. Evidence Synthesis

Because the included studies differed substantially in organisms, tissues, injury models, experimental methods, and outcome measures, statistical meta-analysis was not considered appropriate. A **qualitative narrative synthesis** was therefore performed.

The evidence was organized around four principal themes:

1. **Blastema formation and cellular origins**
2. **Cellular plasticity and dedifferentiation**
3. **Epigenetic reprogramming and chromatin dynamics**
4. **Positional information, differentiation, and tissue restoration**

Comparative synthesis was subsequently performed to identify **conserved mechanisms, model-specific mechanisms, areas of agreement, contradictory findings, and major knowledge gaps**.

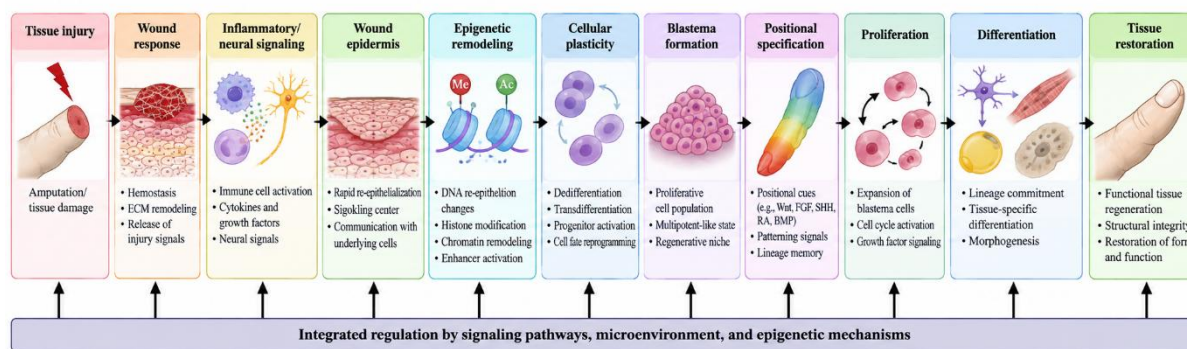
3.15. Comparative Framework

The final synthesis compared regenerative models according to the following dimensions:

Parameter	Axolotl/Newt	Zebrafish	Planarian	Mammalian digit
Blastema formation	Strong	Strong	Blastema-like regenerative tissue	Restricted
Major cellular source	Stump cells/progenitors	Lineage-associated cells	Neoblasts	Local progenitors
Dedifferentiation	Prominent	Partial	Limited/alternative strategy	Restricted
Cellular plasticity	High	Moderate–high	Very high	Moderate
DNA methylation	Important	Dynamic/stable components	Regulatory	Emerging
Histone regulation	Important	Important	Important	Emerging
Chromatin remodeling	Important	Strong evidence	Important	Emerging
Positional information	Critical	Critical	Critical	Critical
Translational relevance	High conceptual value	High	Moderate	Very high

3.16. Conceptual Integration

Finally, the findings were integrated into a unified mechanistic framework describing the relationship between injury and regeneration:



In this context, epigenetic reprogramming was seen as a possible regulatory mechanism between the extracellular environment's injury signals and the intracellular environment's changes in cellular identity. The concept of controlled cellular plasticity was emphasized, in which cells are temporarily made into plastic cells with regeneration competence, but still have enough lineage and positional information to accurately reconstruct tissues.

4. Cellular and Molecular Mechanisms of Blastema Formation

4.1. Injury Sensing and Early Wound Response

The beginning event for tissue injury is what turns a normally differentiated tissue into a regeneration-competent environment (Seifert & Muneoka, 2018). After amputation or severe tissue damage, cells sense mechanical disruption, changes in the ECM, the changes in ionic gradients, the release of intracellular molecules and the damage-associated molecules. These events lead to rapid transcriptional and signaling responses including inflammatory mediators, growth factors, reactive oxygen species, and developmental pathways. Regenerative organisms have tissue protection responses to wounds that rapidly switch from tissue protection to developmental competence, in contrast to the more typical mammalian wound response (Chen & Poss, 2017). For instance, in axolotl limbs the closure of wounds is fast, and a specialized wound epithelium forms to interact with the underlying tissues to establish the early blastema (Scott et al., 2022). Neural signals are also carried to the wound environment that are necessary for moving towards regeneration. Interactions between nerves and wound epithelium have been proven to play a particularly important role in the initiation of regenerative responses in studies that have employed accessory limb models (Karabinis, 2024). Other factors that are important in establishing the regenerative environment include those of the FGF signaling pathway, extracellular-matrix remodeling, and matrix metalloproteinase activity. In this sense, the process of sensing injury should be considered not only as the starting point for tissue repair, but as the first step in a complex information processing process that determines the fate of tissues towards regeneration, remodeling or fibrosis.

4.2. Wound Epidermis Formation

Formation of a specialized wound epidermis is one of the earliest and most important events in epimorphic regeneration. After amputation, the cells on the epidermis move quickly to cover the wound and form a protective layer of epithelium (Brewer, 2018). In amphibians with high regenerative capacity, this structure later on forms an epithelial cap that becomes a specialized signaling centre, called the apical epithelial cap. Conventional wound epithelium is different because the epidermis of the wound is an active communicator with underlying mesenchymal and progenitor populations and supplies the signals that are necessary for the establishment, proliferation and pattern formation of blastema (Gumbry, 2017). The salamander has a special significance for neural innervation of the wound epidermis as a key component in the development and maintenance of the regeneration permissive state of the epidermis. Experimental studies revealed that the epidermis of wounds is not just a passive component of a regenerative niche, but it is instructive. It is involved in growth factor signaling, affects cellular recruitment and contributes to maintenance of undifferentiated blastema cells (McCarthy, 2017). The wound epithelium also undergoes changes at the epigenetic level. For axolotl regeneration, it has been shown that nerve-dependent regulation of DNMT3A and DNA methylation are

related to the formation of the regenerative wound epithelium, so that wound-epithelial formation is directly related to epigenetic regulation. This means the wound epidermis is a bridge that connects wound signals, neural input, extracellular signals and epigenetic changes needed for the blastema to form (Brito, 2018).

4.3. Inflammatory and Immune Responses

Although a very important part of the regeneration process, the result of inflammation is much dependent upon its intensity, duration and type of cells involved. Immediately after injury, immune cells are recruited to clear away damaged material, regulate microbial invasion and begin tissue remodeling. In regenerative species, this inflammatory response is orchestrated with pathways that promote progenitor activation and tissue regeneration instead of chronic fibrosis. Macrophages play a specific role in regulating the availability of growth factors, extracellular-matrix remodeling, cell survival and inflammation resolution. In salamanders, it has been shown that the absence of macrophages can have dramatic effects on the ability of limbs to regenerate, highlighting the importance of immune cells as active players in the mechanism of regeneration rather than as mere traps for pathogens. In mammals, there are also differences in inflammatory and immune responses between regeneration and fibrosis (Popsuishapka et al., 2018). A high and sustained inflammatory signal can activate fibroblasts and lead to deposition of extracellular matrix, which is detrimental to cellular plasticity and growth. The immune environment thus seems to have a well-regulated shift from activation to reconstruction. The microenvironment may modulate local cells' ability to maintain or gain regenerative potential through cytokines, chemokines, signals from macrophages, and stromal cells. Therefore, inflammation may not always be a bad thing for healing, but rather inflammation at the right time may be a necessary component for the formation of a blastema, while inflammation that is out of balance can drive healing towards fibrosis and chronic structural remodeling (Rink, 2018).

4.4. Neural Regulation of Regeneration

Neural regulation is a characteristic of appendage regeneration in salamanders. Nerves that reach into the affected area give signals after limb amputation which are crucial for the development and upkeep of the blastema. The outgrowth of nerves is found to play a more significant role in regeneration than is traditional supposed; denervation not only slows down or even blocks normal outgrowth but also results in fewer neurons than normal. Neural signals communicate with the epidermis and the mesenchymal cells underneath the wound and create a regeneration-permissive environment (Lund-Ricard et al., 2020). The apical epithelial cap and nerve fibers constitute a functional signaling unit which is necessary for blastema proliferation and pattern formation. This interaction has been attributed to molecules such as neurotrophic factors, FGF-related factors, and other nerve-related molecules. Notably, the regulation of the nervous system also seems to link extracellular signaling to epigenetic reprogramming. Regenerative changes in axolotl limbs involve the expression of nerve-dependent changes in the expression of DNMT3A in the early regenerative response, and HDAC-associated mechanisms have been implicated in blastema formation. This implies that signals sent by the nervous system are regulating cellular identity via not only the traditional growth factor pathways but also through chromatin regulation. The accessory limb model has been very helpful in establishing the molecular environment for ectopic regenerative growth requires nerve input (Markitantova & Grigoryan, 2023). In this regard, nerves can be regarded as part of the regenerative signaling network where their roles include coordinating the formation of the wound epidermis, cellular plasticity, proliferation and epigenetic remodeling.

4.5. Recruitment and Activation of Progenitor Cells

Blastema development involves accumulation and activation of cells that are able to proliferate and later give rise to regenerated tissues. Cellular sources differ significantly from one organism to another with regard to regeneration. The populations of mature cells in the connective tissue and other stump-derived cells, in salamanders, can be subjected to significant transcriptional changes and can give rise to blastema formation (Barghouth, 2019). In zebrafish fins, there are multiple populations in a blastema that are lineage associated and yet show considerable lineage restriction. Planarians have another strategy in which the existing neoblast stem cells can multiply and differentiate in response to positional information. Distal amputation also triggers activation and proliferation of local progenitor populations in mammalian digit regeneration. Mammalian digit regeneration also requires activation and proliferation of local progenitor populations after distal amputation. Thus, recruitment is a process that includes both cell movement towards the lesion site and molecular changes

of resident cells to become regeneration-competent cells. This process is mediated by extracellular-matrix remodelling, growth factors, chemokines, neural signals and wound-epithelial factors. Cellular activation is accompanied by changes of proliferation, metabolism, transcription and chromatin accessibility. Importantly, complete dedifferentiation is not a requirement for progenitor recruitment. Rather, cells can acquire, temporarily, greater plasticity, while still carrying with them information from their lineage (Gumbrys, 2017). The zebrafish is a very good model for this, as the chromatin landscape is dynamic during regeneration, while DNA methylation of lineage specific sequences is more stable. Thus, the activation of the progenitors is not just the production of an undifferentiated cell pool (Chen & Poss, 2017).

4.6. Cellular Origins and Heterogeneity of the Blastema

All the classical ideas of the blastema as a uniform mass of undifferentiated cells have been significantly modified by lineage tracing and single cell technologies. Recent studies suggest that blastemas have multiple cellular and developmental populations with different molecular signatures and regenerative abilities. Connective-tissue populations can become progenitor-like in axolotl limbs, while populations of the epidermis, immune system, vascular system, and others are found in different molecular compartments. Different types of cells in the mature stump participate in the formation of the developing blastema at various times, and some are used as pattern-forming populations, while others are mainly used as following established positional cues. There is additional evidence of lineage restriction in the regeneration of zebrafish fins: different populations of cells can give rise to regeneration, and some cells in the population retain some aspects of their original lineage identity. This shows that it is not necessary for the cells to be completely reprogrammed in order to achieve high regenerative capacity. Other organisms, such as planarians, have another organization in which pluripotent stem cells exist along with specialized stem cells and differentiated positional-information cells. So, blastema heterogeneity is due to both cell origin and functional specialization. The ability to resolve these populations is gained by the growing importance of single-cell RNA sequencing and spatial approaches. Knowing about blastema heterogeneity is crucial since regeneration depends on interactions between multiple cellular compartments and is not solely a function of a single universal population of stem cells. Thus, the blastema can be viewed as a dynamic multicellular regenerative ecosystem.

4.7. Blastema Proliferation and Growth

When a blastema becomes regeneration competent, it is possible to control its growth and increase the number of cells needed to restore the lost structure. Positional information, metabolic programs, interactions between growth factors and developmental signaling pathways, and extracellular-matrix components all contribute to blastema proliferation. During the various stages of regeneration, a variety of signaling systems are involved in the maintenance of the same states of proliferation and progenitor cells, including FGF, Wnt, BMP, and others. But, proliferation is not a complete explanation for successful regeneration since unchecked proliferation would not bring back the proper anatomy. The growth of blastemas is accordingly regulated both in space and in time, with cells in increasingly specialized ways reacting to patterning signals and initiating differentiation pathways. Salamanders form a blastema under the wound epidermis which grows with the morphologically specified regenerate. Blastema cells also proliferate in zebrafish fins and then gradually contribute to the fin rays and tissues. An extra layer of control over proliferation is provided by epigenetic regulation. The chromatin remodeling complexes and histone modifying enzymes regulate programs of transcription for cell cycle progression and subsequent differentiation. It is thus crucial to precisely coordinate proliferative state maintenance with a limitation on indefinite self-renewal during regeneration (Chen & Ding, 2022). One of the hallmark traits of regeneration is thus the capacity to temporarily allow cells to enter a state of proliferation and gradually shut themselves down as they begin to assume differentiated identities. This change is essential for the prevention of overgrowth, and adequate production of cells is essential for anatomical reconstruction.

4.8. Positional Information and Pattern Formation

Proper regeneration involves not only cell replication, but also the recognition of missing structures and their location. During the process of pattern formation, the biological coordinates of positional information guide blastema cells. Salamander Limbs have cells that encode information along anatomical axes such as proximal-distal and anterior-posterior identities. This information is understood during regeneration and in some cases, is even reprogrammed, to assure proper reconstruction. Positional information in planarians is retained mainly by

the exchange of signaling molecules from the differentiated muscle cells. After injury, positional information is quickly reset and then directs pluripotent neoblasts to correct tissue identities. This shows that regeneration is driven not only by the cellular potential, but also by spatial instructions (Du et al., 2024). Different developmental pathways (e.g., Wnt, FGF, SHH, BMP, and retinoic-acid signaling) help to establish patterns in various organisms. Importantly, information on position is linked to cellular epigenetic states for activation of the correct developmental programmes at the right anatomical place. Positional signaling errors can lead to abnormal or duplicated structures even when there is good cell proliferation (Sands et al., 2024). Hence, blastema formation is a preliminary step in regeneration, and the recovery of tissues requires a later information-processing phase, where cells become aware of their position and arrange for cell growth, movement, differentiation and morphogenesis.

4.9. Differentiation and Tissue Restoration

Epimorphic regeneration culminates in successive morphogenesis of blastema-derived cells, followed by a reconstruction of the mature tissues. In early stages of regeneration, cells need to be sufficiently plastic to respond to patterning signals, but as regeneration progresses cells increasingly adopt the identities of their tissue of origin (Llewellyn et al., 2024). It includes coordinated shifts in the activity of transcription factors, the accessibility of chromatin, cell-cycle status, extracellular-matrix interactions, and developmental signaling. Blastema cells in salamander limbs are responsible for creating cartilage, muscle, connective tissue, vasculature, epidermis and more in a spatial and temporal order and context. The regeneration of the fins in the zebrafish also proceeds via the differentiation into osteoblasts, epidermal cells, populations associated with fibroblasts and others. Regeneration of mammalian digits has been found to reactivate parts of developmental gene-regulatory programmes, including genes involved in the development of the skeleton and morphogenesis of the limbs. Therefore, tissue repair is not just cell replacement, it is a partial reversion into development. This is especially when it comes to epigenetic mechanisms as during this transition the chromatin states associated with plasticity and proliferation need to be reconfigured to establish stable differentiated identities. Regeneration thus ends with a gradual decrease in cellular plasticity (Aloia et al., 2019). The newly formed tissues need to develop permanent molecular and structural properties and to be fully functional in relation to the adjacent tissues. Successful differentiation, therefore, is the culmination of a series of events that start with injury sensing, followed by wound signaling, blastema formation, patterning, proliferation, and finally the return to mature tissue architecture.

5. Cellular Plasticity and Epigenetic Reprogramming During Regeneration

5.1. Cellular Plasticity in Regenerative Biology

The ability of differentiated or progenitor cells to change their molecular identity, functional state, or developmental potential in response to environmental signals is called cellular plasticity. It's a key element of regenerative biology because often, tissue repair requires cells to alter their behavior after injury. However, in highly regenerative organisms, mature cells can be more malleable, multiply, migrate and then regenerate new tissues (Huyghe et al., 2024). But in general, it's not necessary to completely transform all cells into pluripotent stem cells. In contrast, cells seem to change their identities in a regulated and context-specific manner. Differentiated connective-tissue populations have been found to be able to reach progenitor-like transcriptional states during limb regeneration in axolotls, and changes in chromatin accessibility have been found to be substantial between differentiated populations of cells with lineage-specific DNA methylation in zebrafish (Nemec & Kilian, 2021). The observations indicate that regenerative plasticity is not achieved by erasure of the epigenetic state of the cell, but rather by selective modification of cell-state programs. Thus, cellular plasticity can be considered as a continuum, from low level changes in gene expression to high level changes such as dedifferentiation and lineage conversion (Basu & Tiwari, 2021). It is regulated by injury, developmental pathways, interactions with the extracellular matrix, transcription factors and epigenetic mechanisms. It is crucial to comprehend this continuum to understand why some organisms are able to regenerate complex structures, while others tend to form scar tissue (Cooke & Lai, 2023).

5.2. Dedifferentiation and Partial Reprogramming

Dedifferentiation is a process whereby differentiated cells lose markers of their differentiated state and gain markers in their progenitor or less differentiated state. It has been traditionally recognized as one of the characteristics mechanisms of salamander limb regeneration. However, recent research has shown that this dedifferentiation is more correctly termed partial or controlled reprogramming (Llewellyn et al., 2024). It is not necessarily the entirety of the developmental history that gets erased—only certain transcriptional and epigenetic programs are altered, to make the cells receptive to signals of regeneration. In axolotl limbs, there are extensive molecular changes in connective-tissue cells that bring them back to progenitor-like states, while lineage information may be relevant to further differentiation. Similar regulation of chromatin accessibility but less dynamic DNA methylation occurs during regeneration in zebrafish. This indicates that dedifferentiation can cause developmental flexibility without eliminating unconstrained pluripotency. In fact, biological benefit of such controlled reprogramming comes from the fact that eradicating all traces of cell identity can disrupt positional information and make it easier to differentiate them into improperly (d'Angelo et al., 2019). Selective access and silencing of developmental regulatory regions by epigenetic mechanisms are likely to be the molecular basis for this balance, while lineage associated marks remain stable. Dedifferentiation should thus be considered as a process of transition between differentiated and regenerative states that is reversible and not, as often has been interpreted, a return to an embryonic state (Torre, 2022).

5.3. Transdifferentiation and Lineage Conversion

Transdifferentiation is the ability to transform a differentiated cell type into another differentiated cell type, either directly or indirectly, without the use of a common pluripotent intermediate step. The study of regeneration has sparked interest in this mechanism since it may offer a way to regenerate specialised cells without the need for transplanting stem cells from outside the body. From the highly regenerating organisms, evidence points towards the developmental re-programming of cells after injury a process that can involve partial transdifferentiation, albeit this is partially model- and tissue-specific. It is generally concluded from the lineage-tracing studies of salamander regeneration that there is a lot of plasticity in the system, but there are also lineage limits, which means that there are intermediate progenitor-like states that would account for the apparent conversion of one cell type to another. Planarians represent an alternative system in which pluripotent neoblasts produce multiple differentiated tissues, instead of primarily through direct transdifferentiation. Interpretation of regenerative cell fate experiments is therefore complicated by the difference between transdifferentiation and dedifferentiation (Rothman & Jarriault, 2019). Epigenetic changes are likely to play a role in lineage conversion by changing the accessibility of chromatin, transcription factor binding and enhancer activity. The cell undergoing lineage conversion should be able to shut down the old transcriptional program and initiate a new one. These transitions can't be a single master gene, they have to be coordinated changes. Therefore, transdifferentiation is a more complex process, comprising of transcription, chromatin remodeling, and extracellular signals, all of which play a role in creating a new cell type (Anatskaya & Vinogradov, 2022).

5.4. Progenitor-State Acquisition

Regenerative cells often enter a short-lived progenitor-like state in which they become more proliferative, undergo changes in transcription, are more responsive to developmental signals, and are less terminally differentiated. This state is important especially because it is known to be the cellular flexibility required for the growth of the blastema without necessarily producing fully pluripotent cells. In the case of axolotl limbs, the development of the progenitor molecular state is observed during regeneration in mature populations of the connective tissue, which tend to approach progenitor-like molecular states during the process (Folguera Blasco, 2019). Different lineages of cells in zebrafish give rise to the "blastema" while retaining lineage restrictions, suggesting that acquisition of the progenitor state can happen in specific cellular domains. In the regeneration of mammalian digits, a blastema also forms, made up of local populations of progenitor cells that are able to regenerate the distal part of the digit. The mechanisms of control of progenitor-state acquisition include extracellular signals, transcription factors, growth pathways, as well as epigenetic mechanisms (Larionov et al., 2024). Regenerative programs should be opened and differentiated ones should be appropriately modulated at chromatin level. Cells also have to be sensitive to positional information in order to differentiate into the appropriate tissue type. The progenitor state is thus not permanent and more specialized in function. Can be considered as an intermediate state between mature differentiation and regenerative reconstruction. The dynamics of cell entry and exit of this state are especially relevant for regenerative medicine applications, as

therapeutic interventions might attempt to recapitulate the controlled plasticity exhibited by cells in their native environment without triggering the unrestricted pluripotency observed in tumor cells (Kumar et al., 2022).

5.5. DNA Methylation and Demethylation

One important epigenetic mechanism that regulates gene activity, cellular identity and developmental stability is DNA methylation. Injury-responsive and developmental genes may be open or closed to the transcription machinery during regeneration due to changes in DNA methylation. Modulation of DNMT3A is also found during early wound-epithelium formation in axolotl limb regeneration, and requires neural signaling (Bhat et al., 2024). The experimental DNA methylation inhibitors, decitabine, changed the expression of genes and cells, as well as restored regenerative properties of wounds with poor regeneration. The results indicate that DNA methylation can be a barrier to regenerative competence. But, there seems to be no genome-wide (generalized) demethylation during regeneration (Zare et al., 2023). Lineage-specific DNA methylation signatures are highly persistent over regeneration in the zebrafish fin, whereas chromatin accessibility is dynamic. Associating enhancers with regeneration also might be kept in a hypomethylated or permissive state prior to injury, allowing for rapid activation upon initiation of regeneration. Therefore, DNA methylation seems to be acting on a selective basis instead of by global epigenetic reset (D'Aniello et al., 2017). This separation is essential as it is possible to keep cell memory when methylation is stable in the lineage, and to restore cells to their original state when methylation changes locally. Thus, methylation of the DNA should be regarded as a dynamic part of the regenerative regulation process, which is responsible for the balance between the plasticity, lineage stability, and developmental competence of cells.

5.6. Histone Modifications and Chromatin Remodeling

Regeneration involves changing the transcriptional response, and chromatin remodeling complexes and histone modifications affect the accessibility of regions in the genome. While generally speaking the acetylation of histones results in a more transcriptionally permissive chromatin environment, the reverse is true for deacetylation, which may help to create a repressive environment, albeit with some highly context-dependent functions in individual histone deacetylase proteins (Aloia et al., 2019). In axolotls, histone deacetylation has been shown to be actively needed in the formation of the blastema and outgrowth of regeneration. However, the components of NuRD chromatin remodeling complex are necessary for proper fin regeneration in zebrafish, suggesting a connection between nucleosome remodeling, histone regulation and blastema proliferation and differentiation. These observations suggest that restoration of chromatin structure must be controlled at specific time points, not just a global activation of open chromatin, for regeneration to occur. Accessibility of injury-responsive and developmental genes is required during early regeneration, while selective repression and activation are required during later stages for establishment of differentiated tissues (Kyriazi et al., 2020). Histone modifications can then serve as molecular switches that control the switching between regenerative states. Additionally, chromatin-remodeling complexes modify nucleosome positioning in order to make certain regulatory regions accessible to action of transcription factors (Baghmaleki, n.d.). Histone modifications and chromatin remodeling act in concert as a mechanistic link between signals from the outside and changes in gene expression. They are likely to be important not only for stage-specific regulation but also for making cellular plasticity transient and regeneration eventually ending in stable tissue differentiation.

5.7. Chromatin Accessibility and Regeneration-Specific Enhancers

Chromatin accessibility is perhaps a most important regulatory aspect of regenerative biology given that cells need to rapidly activate genes that may be comparatively inactive in mature tissues. After injury, certain regions of the genome are made accessible to transcription factors, which can turn on developmental and injury-response programs. Zebrafish fin regeneration and the epithelium's capacity to regenerate appendages is good evidence for this mechanism. Epigenomic studies have revealed extensive chromatin accessibility changes during regeneration and comparatively stable DNA methylation in a lineage. The regeneration-specific enhancer elements have been identified near genes that are turned on after damage, and are often referred to as tissue regeneration enhancer elements. Evidence that non-coding regulatory DNA actively regulates regenerative transcription, and not just reflects it, has been provided by functional studies, which showed that several such enhancements can induce expression of regeneration-associated genes. These enhancers could be dormant developmental regulatory elements waiting to be turned on in the event of damage. Through selective activation,

differentiated cells can gain access to developmental programs without having to undergo full genomic reprogramming. Accessibility of chromatin is thus a key mechanism by which injury signals can rapidly impact cellular behavior. Identification of regeneration-specific enhancers also has translational relevance as these elements may then be used to direct therapeutic genes selectively when tissue is being repaired. The activation of enhancers, the binding of transcription factors to enhancers, and the way that epigenetic mechanisms control the accessibility of enhancers continue to be a key research priority.

5.8. Non-Coding RNAs and Epigenetic Regulation

The non-coding RNAs are another level of regulation in regenerative biology. Long non-coding RNAs, microRNAs, circular RNAs, and small RNA pathways may control the expression of genes either at a posttranscriptional level or at a level that influences the state of the chromatin and the state of the cell. Small RNA pathways, including PIWI-associated pathways, play a role in the maintenance of stem-cell pools, genome stability and differentiation in stem-cell rich organisms like planarians. During vertebrate regeneration, proliferation, apoptosis, differentiation and remodeling can also be regulated by microRNA networks. Non-coding RNAs can flexibly coordinate the activities of groups of genes instead of regulating individual targets independently, as the transitions between the states of cells require rapid changes, and non-coding RNAs provide such flexibility. They can also bind to transcription factors and proteins that modify the chromatin, which leads to feedback loops that maintain regenerative states. Furthermore, non-coding RNAs can modulate the extracellular signaling and communication between cells in the regeneration microenvironment. The functional evidence is not consistent among species, but literature has indicated that non-coding RNAs play a part in fine regulation of cellular plasticity and tissue restoration (Chen & Ding, 2022). To determine whether small-RNA sequences are the true regulators of gene transcription, or simply by-products of transcription, future studies will need to combine transcriptomics, small-RNA sequencing, chromatin profiling and functional perturbation. This type of work could uncover other mechanisms by which regeneration regulates proliferation, differentiation, positional information, and epigenetic memory.

5.9. Epigenetic Memory and Lineage Stability

Epigenetic memory describes the ability of molecular information to remain even after major changes in the transcriptional state of the cell, as a result of a previous developmental history. This is a more and more significant notion for regeneration as cells need to be plastic enough to be involved in the regeneration of tissues yet carry information that facilitates tissue-specific differentiation in the proper manner. This is especially true for the regeneration of fins in the zebrafish. Even though substantial changes in chromatin accessibility and gene expression occur, DNA methylation signatures are rather stable throughout the process of regeneration between the stages in the same lineage. This suggests that cells don't lose their identity when they activate these programs to regenerate. These ideas have also been suggested by the studies of axolotls where separate populations of mature cells are isolated and seeded to contribute blastema, but retain memory of their developmental history. Epigenetic memory may thus represent a protection against the uncontrolled conversion of the cells. It enables a cell to temporarily switch to a different developmental program while keeping track of its original lineage at the molecular level. This balance could be crucial for the proper regeneration, as the regenerated tissues should maintain the appropriate cellular composition and structure. The loss of lineage memory might potentially also enhance plasticity, though at the same time reduce pattern fidelity and tissue organization (Saraswathibhatla et al., 2023). Thus, it is important to note that epigenetic memory need not be seen as a barrier to regeneration, but could be a necessary part of the effective regeneration/reprogramming process (Theocharis et al., 2019).

5.10. Plasticity–Stability Balance

For successful regeneration, there should be a dynamic balance between cellular plasticity and cellular stability. If the stability is too high, this would be detrimental to the ability of differentiated cells to respond to injury, while if the stability is too low, inappropriate lineage conversion, uncontrolled proliferation, and loss of tissue organization could occur (Llewellyn et al., 2024). Transient and localized cellular reprogramming seems a solution to this problem in the case of regenerative organisms. In early regeneration, injury signals stimulate chromatin remodeling, developmental gene activation and gain of progenitor-like characteristics. With the emergence of pattern, the blastema gradually loses its plasticity and enters into the tissue-specific differentiation program. This transition could involve stable DNA methylation and positional information, as well as lineage-

associated transcriptional networks (d'Angelo et al., 2019). In zebrafish, this is clearly seen as a dynamic shift in chromatin accessibility, while lineage-specific methylation is stable. In the axolotls, several populations of mature cells can give rise to the blastema without having a universal pluripotential capacity. The results indicate that the process of regeneration does not involve the complete erasure of identity of differentiated cells, but controlled de-differentiation. The plasticity-stability balance has significant consequences in regenerative medicine. Ideally, therapeutic reprogramming should mimic the temporary regenerative window not to permanently deprogram the cell to a highly plastic state. It will take careful control of epigenetic factors, signaling pathways and the tissue environment to achieve such control. Manipulating this balance is likely to be the determining factor between successful conversion of endogenous mammalian cells into regeneration-competent populations and failure (Folguera Blasco, 2019).

6. Signaling, Microenvironment, and Comparative Regeneration Modalities

A complex network of developmental signaling pathways, cell-to-cell interactions, epigenetic mechanisms, and extracellular-matrix (ECM) conditions control the regeneration of injured tissues, leading to either functional restoration or incomplete repair with fibrosis (Velikic et al., 2024). Wnt/ β -catenin signaling is one of the important signaling pathways involved in tissue regeneration, stem-cell function, proliferation, and developmental patterning. Wnt activity is activated in wound associated tissue after injury and plays a role in the proliferation and specification of the regenerative populations. In planarians, Wnt signaling is also crucial for anterior–posterior polarity and injury-induced Wnt activation promotes posterior identity. Wnt also works in concert with other pathways, such as fibroblast growth factor (FGF) and bone morphogenetic protein (BMP), to regulate blastema formation and patterning in vertebrate regeneration. The FGF signaling is similarly related to blastema development, cellular proliferation, and tissue organization (Xue et al., 2024). Salamanders establish FGF-related signals within the wound epidermis, nerves and mesenchymal tissues that maintain the proliferative and developmentally competent state of blastema cells. The FGF-associated regulatory networks are also involved in zebrafish regeneration, including the activity of enhancer genes in regeneration. Positional identity, proliferation, differentiation, ECM remodeling, and pattern formation are further important aspects of developmental identity controlled by other pathways such as Sonic hedgehog (SHH), BMP/TGF- β , Notch, and retinoic acid. The influence of positional identity, in particular, makes retinoic acid an important factor in salamander limb regeneration, while the role of BMP/TGF- β signaling can be context-dependent in some cases, promoting regeneration, and in others, causing fibrotic responses. These pathways are not separate signaling pathways, but rather a coordinated regulatory network that is able to simultaneously sense information regarding injury and development.

Signaling and epigenetic mechanisms play an important role in explaining the intertissue and interspecies differences in regenerative capacity. Finally, the activities of developmental signals (e.g., Wnt, FGF, SHH, BMP/TGF- β , Notch, and retinoic acid) are mediated by regulatory factors that rely on accessible chromatin and appropriate gene-regulatory environments. On the other hand, DNA methylation, histone modifications, chromatin remodeling, and accessibility of enhancers dictate the ability of injured cells to effectively respond to extracellular signals. So it's not enough to simply switch on a developmental pathway to get regeneration to happen if the genes activated by that pathway are epigenetically silenced. Regenerative organisms seem to be able to specifically change the chromatin but still maintain key lineage-associated information. The Zebrafish fin regeneration is an exceptionally informative example, with a dynamic chromatin accessibility profile occurring during regeneration, but a relatively stable DNA methylation profile for each lineage. This is in line with the idea of controlled cellular plasticity, instead of erasure of cellular identity. Additionally, in axolotls, extracellular signals can modulate epigenetic changes in the regenerative wound epithelium, illustrating the interplay between signaling and epigenetic states. The Hippo/YAP pathway also represents another important connection between tissue environment and cell behaviour since YAP is sensitive to cell density, mechanical forces, ECM stiffness, and biochemical signals. These properties allow Hippo/YAP to modulate proliferation, survival, progenitor-state maintenance and differentiation. Therefore, regeneration needs to be considered and analysed as a systems-level process where extracellular signals, chromatin state, cellular identity, developmental pathways, and tissue mechanics continuously play against one another.

Both the regenerative microenvironment is important and blastema cells cannot work in isolation from surrounding tissues. The wound epidermis, nervous system, immune cells, fibroblasts and ECM create a

specialized developmental niche that controls cellular proliferation, migration, plasticity, positional information and differentiation. After injury, the wound epidermis quickly forms and integrates with the nests of proliferating progenitors, whereas nerves give trophic and developmental signals to enable effective regeneration. Immune cells produce inflammatory mediators, phagocytose damaged material and control matrix remodeling. The interaction between nerves and wound epidermis is especially crucial during salamander limb development for the development of the specialized epithelial signaling center required for blastema formation. Macrophages are also involved in the shift from inflammatory injury responses to regenerative remodeling. It is important, therefore, to treat the ECM as more than just a scaffold, but as a signaling molecule as well. It is composed and has mechanical properties which affect cellular behaviour, growth factor availability, mechanotransduction and may affect chromatin regulation. Fibroblasts play a vital role as they are capable of changing their morphology to a regenerative type or profibrotic depending on the tissue environment. Recent data from digit regeneration in mice and rats suggest that the areas of tissue that regenerate have less stiffness and less collagen, while those that don't regenerate have more. Recent work on digits regenerating in mammals has shown that non-regenerating areas are stiffer and collagen-rich, but regenerating areas are softer with more hyaluronic acid. These variations indicate that the mechanical properties of the ECM could dictate whether damaged tissues are allowed to regenerate or fibrose. Thus, changing the matrix-associated conditions might serve as a strategy for changing the behavior of fibroblasts and thus, enhance their regenerative competence.

Conserved principles of cell signaling and the microenvironment can be achieved through very different cellular strategies, as shown by comparative regeneration models. Vertebrate epimorphic regeneration is a classic phenomenon of mature cell contribution after amputation giving rise to a proliferative blastema, as seen in axolotls and newts (Du et al., 2024). There is significant molecular reprogramming of connective-tissue populations, and the progenitors have some elements of the developmental identity. Positional information in the resulting blastema is then used to differentiate skeletal, muscular, connective, vascular and epithelial tissues. Zebrafish fin regeneration also involves the formation of the wound epidermis and blastema, but as cells undergo significant changes in transcription and chromatin accessibility, they can also undergo changes in lineage-associated DNA methylation (Saraswathibhatla et al., 2023). Selective activation of developmental regulatory networks enables to balance the expression of injury-responsive genes, while allowing for the activation of regeneration-specific enhancers, revealing that activation of developmental regulatory networks can be sufficient to restore large amounts of tissue without dedifferentiation. In contrast, planarians are used, and have generously been observed to have adult stem cells (neoblasts) that are capable of generating many different cell types, including pluripotent cells (Sands et al., 2024). They require highly plastic stem cells and positional information from differentiated tissues, especially muscle cells to regenerate. The regeneration of the tips of the digits in mammals is a more limited form of epimorphic regeneration that shows that mammals are capable of regeneration in certain anatomical contexts. Surgery of the distal digits tends to produce blastema formation and restoration of tissue, while more proximal injuries will lead to fibrosis. Reactivation of developmental programs and different types of fibroblasts are revealed by transcriptomic and single-cell analyses, and the composition of ECMs and the mechanics of tissues further separate regenerative and non-regenerative environments.

When all of this is considered together, comparative analysis shows that there are mechanisms conserved across species, and species-specific mechanisms at play in epimorphic regeneration. Many of the processes involved in injury sensing, wound signaling, progenitor activation, developmental pathway reactivation, positional information, controlled proliferation, and differentiation are broadly conserved in regenerative organisms. However, the cellular origin and plasticity are very variable. It is important to note that there is significant lineage restriction among mature stump-derived cells in Salamanders, a high degree of lineage restriction with dynamic chromatin remodeling in zebrafish, and lineage restriction and reduced regenerative potential in specialized tissues in mammals. Shared epigenetic mechanisms such as DNA methylation, chromatin accessibilities, histone modifications, and non-coding RNAs, are also shared, but have distinct temporalities and functional relevance. One important common principle is the need to coordinate between cellular plasticity and maintenance of lineage identity. The local microenvironment consisting of epithelial, neural, immune, fibroblast and ECM components is also critical. Species differences are thus not necessarily a sign of the lack of regenerative pathways in mammals but may also reflect the difference in the way these pathways are activated, coordinated, maintained and terminated. Comparative studies then offer a roadmap for uncovering shared

mechanisms of regeneration, and for understanding how signaling during development, epigenetic regulation, cellular plasticity and tissue mechanics might ultimately be targeted to encourage regeneration without aberrant reprogramming, abnormal patterning or fibrosis (Theocharis et al., 2019).

7. Regeneration, Fibrosis, and Translational Perspectives

Regeneration and fibrosis represent two fundamentally different outcomes of tissue injury, with regeneration restoring tissue architecture through coordinated proliferation, pattern formation, differentiation, and remodeling, whereas fibrosis primarily restores structural integrity through extracellular-matrix deposition and scar formation. Mammalian digit-tip regeneration provides an important model because distal digit injury can produce regenerative repair, while more proximal injury generally results in fibrosis. This distinction indicates that regenerative competence is strongly influenced by local cellular and microenvironmental conditions. Regenerative tissues establish a blastema-like population and reactivate developmental gene networks, whereas fibrotic tissues exhibit persistent inflammation, profibrotic fibroblast activation, collagen accumulation, altered tissue mechanics, and failure to adequately reactivate developmental programs. The extracellular matrix is particularly important in this distinction, as regenerative digit regions have been associated with softer, hyaluronic-acid-rich environments, whereas non-regenerative regions display increased stiffness and organized collagen deposition. Consequently, regeneration and fibrosis should not be viewed simply as different degrees of wound healing but as distinct tissue-level responses to injury. Epigenetic mechanisms may contribute to this divergence by regulating whether differentiated cells maintain stable identities or acquire regenerative plasticity. DNA methylation, repressive histone modifications, chromatin compaction, and enhancer accessibility can potentially restrict the activation of developmental programs in mammalian tissues. Evidence from axolotl and zebrafish regeneration indicates that DNA methylation and dynamic chromatin accessibility participate in establishing regeneration-competent cellular states, suggesting that some mammalian cells may retain latent regenerative programs but fail to activate them efficiently following injury.

The limited regenerative capacity of mammals is unlikely to result from a single molecular deficiency; rather, it reflects the combined effects of cellular, molecular, epigenetic, inflammatory, and microenvironmental constraints. Mammalian tissues such as liver, skin, skeletal muscle, bone, and the distal digit retain varying degrees of regenerative potential, indicating that endogenous regenerative mechanisms remain present but are differentially regulated. In the mouse digit-tip model, local progenitor populations can form a blastema and transiently activate developmental programs associated with skeletal development and limb morphogenesis, whereas non-regenerative injuries fail to produce equivalent developmental responses. Differences in fibroblast populations, extracellular-matrix composition, inflammation, and tissue mechanics further contribute to these divergent outcomes. These findings suggest that enhancing mammalian regeneration may require modification of the endogenous tissue environment rather than reliance solely on transplantation of exogenous stem cells. Regeneration-specific enhancer elements provide one potential route for selectively activating regenerative programs. Studies in zebrafish have identified tissue regeneration enhancer elements that become accessible following injury and regulate genes associated with regenerative responses, including *fgf20a*, *mdka*, and *cx43*. Such regulatory elements could potentially be incorporated into injury-responsive therapeutic systems to activate genes selectively at damaged sites while minimizing constitutive expression. However, regulatory elements identified in highly regenerative species cannot automatically be assumed to function identically in humans, and comparative genomic and functional studies are required to establish their conservation and therapeutic relevance.

Controlled epigenetic reprogramming represents another promising strategy for enhancing regeneration. Unlike conventional induced pluripotency, the objective of regenerative reprogramming is not to erase cellular identity completely but to establish a temporary, spatially restricted, progenitor-like state that enables tissue reconstruction while preserving lineage information. DNA methylation inhibitors, histone-modifying enzymes, and chromatin-remodeling mechanisms can influence regenerative responses, but their effects depend strongly on tissue type, timing, and dosage. Consequently, therapeutic manipulation of the epigenome must be precisely controlled to prevent inappropriate proliferation, abnormal differentiation, or loss of cellular identity (Chen & Ding, 2022). Endogenous cellular reprogramming may be particularly valuable because it seeks to activate resident cells within injured tissues rather than introduce external stem cells. Evidence from axolotl regeneration demonstrates that mature stump-derived populations can acquire progenitor-like properties, while mammalian

digit regeneration shows that local cells can participate in blastema formation. Combining transcription-factor modulation, epigenetic regulation, growth-factor delivery, extracellular-matrix engineering, and immune modulation could therefore provide a multifactorial strategy for inducing regenerative competence. Stem-cell and tissue-engineering approaches can complement these strategies by providing replacement cells together with biomaterials, growth factors, mechanical cues, and controlled differentiation signals (Hora & Wuestefeld, 2023). Importantly, blastema biology suggests that successful tissue engineering should recreate aspects of the dynamic regenerative niche rather than simply deliver stem cells. Biomaterials capable of controlling stiffness, degradation, ligand presentation, and growth-factor release may help reproduce the environmental conditions required for cellular plasticity, proliferation, positional specification, differentiation, and tissue maturation.

Immune modulation, extracellular-matrix regulation, and emerging multi-omics technologies provide additional opportunities for understanding and enhancing regeneration. Appropriate immune activation is essential for regenerative repair, whereas chronic inflammation can promote fibrosis through persistent fibroblast activation and extracellular-matrix deposition. Macrophages, fibroblasts, matrix components, and mechanical properties therefore represent active regulators of regenerative cell fate rather than secondary consequences of injury. Single-cell RNA sequencing, single-cell ATAC-seq, DNA methylome profiling, and spatial transcriptomics are increasingly enabling researchers to resolve blastema heterogeneity, reconstruct cell-state trajectories, identify chromatin changes, and determine the spatial organization of regenerative programs. Integration of these datasets may clarify how cellular identity, epigenetic state, positional information, and microenvironmental signals interact during regeneration. CRISPR-based epigenome editing further provides a potential means of manipulating specific enhancers, promoters, DNA-methylation states, or histone modifications without globally altering the genome sequence. Nevertheless, translation from salamanders, zebrafish, and planarians to humans remains challenging because regenerative outcomes depend on interconnected cellular, developmental, immune, mechanical, and epigenetic processes. Increased cellular plasticity and proliferation may also introduce risks of abnormal tissue growth, tumorigenesis, altered cellular identity, and genomic instability. Therefore, future regenerative therapies will likely require local, temporary, and highly targeted interventions that combine epigenetic regulation with extracellular-matrix modification, immune modulation, developmental signaling, and activation of endogenous progenitors. Long-term studies will be essential to determine whether enhanced regeneration can be achieved while preserving tissue identity, genomic integrity, and normal mechanisms of cancer surveillance.

Discussion

This systematic review shows that blastema formation and cellular plasticity during epimorphic regeneration are orchestrated by an integrated process between cell identity, injury responsive signals, epigenetic remodeling and the regenerative microenvironment. The evidence from salamanders, zebrafish, planarians and mammalian models suggests that the blastema is not composed of a uniform pool of undifferentiated stem cells, but rather a non-uniform, dynamically organized population of lineage-associated progenitors, dedifferentiated cells, and locally activated resident populations. It has been shown that differentiated, stump-derived cells can gain progenitor-like properties in salamanders (especially axolotls and newts), whereas zebrafish regeneration shows significant changes in chromatin accessibility with relatively stable DNA methylation in lineage-specific regions, suggesting controlled, but not complete, epigenetic reprogramming. This difference is relevant because this can mean that cells are able to become sufficiently "plastic" enough to regenerate without losing their original identity. Epigenetic events seem to serve as a molecular link between the extracellular injury signals and these changes in the states of cells. Regenerative competence is selectively regulated by DNA methylation and developmental and injury-responsive genes are regulated by changes in chromatin structure and remodeling complexes in a stage-dependent manner. Further, non-coding regulatory regions that can reactivate developmental programs of transcription without genome resetting are specific to regeneration. They act in concert with Wnt/ β -catenin, FGF, SHH, BMP/TGF- β , Notch, RA, Hippo/YAP, neural signaling, immune activation and extracellular-matrix remodeling pathways to regulate blastema proliferation, patterning, differentiation, and tissue repair. The relative low regenerative response of mammals, especially in cases of digit-tip regeneration, indicates that regenerative competence is more than just absent, but is also limited by factors such as inflammatory, epigenetic, cellular and microenvironmental. Thus, therapeutic approaches should seek to temporally and spatially restricted reprogramming, without the aim of dedifferentiation. These may

include epigenetic modulation, activation of enhancers, delivery of growth factors, engineering of extracellular-matrix via biomaterial and immune regulation. Some differences in species and humans, however, do need to be carefully functional validated, and emerging single-cell multi-omics, spatial profiling, and CRISPR-based epigenome editing, may be useful in identifying safe and precise mechanisms to improve endogenous regeneration.

Conclusion

Finally, this systematic review showcases that epimorphic regeneration is a highly coordinated, multistage process that involves interplay between injury responses, cellular plasticity, blastema formation, developmental signaling, epigenetic regulation, and the regenerative microenvironment. Based on data from the axolotl, newt, zebrafish, planarian and mammalian models, the formation of the blastema does not rely on a single universal population of stem cells but, rather, on lineage-associated progenitors, dedifferentiated cells, local resident stem cells, and locally activated populations. The ability of cells to be "plastic" is best viewed as a controlled and reversible process, during which differentiated cells take on progenitor characteristics, but still maintain some degree of lineage and positional memory. Epigenetic events such as DNA methylation, histone modifications, chromatin remodeling, activation of enhancers specific to regeneration and the regulation by non-coding RNAs offer a major molecular link between signals of the injury and changes in cellular identity. The evidence is especially in favour of dynamic chromatin accessibility and selective epigenetic remodeling and not the erasure of the cellular identity. Overall, the comparative results also reveal that the Wnt/ β -catenin, FGF, SHH, BMP/TGF- β , Notch, RA, Hippo/YAP, neural signaling, immune responses and extracellular-matrix interactions all influence regenerative competence. Importantly, limited mammalian regeneration indicates that endogenous regenerative programs might still be present but are limited by epigenetic, inflammatory, cell, and microenvironmental barriers. Thus, the key novel strategies of epigenetic control, enhancer-specific to regeneration, extracellular-matrix engineering, immune modulation and targeted signaling modulation would be promising strategies to pursue in regenerative medicine. The translation of mechanisms observed in highly regenerative organisms to humans, however, needs to be carefully validated as overplasticity could lead to abnormal cellular proliferation, inappropriate differentiation, or loss of tissue identity. Combining these techniques with lineage tracing, single-cell multi-omics, spatial profiling, and functional epigenetic perturbation will be key to defining safe strategies that promote enhanced endogenous tissue regeneration and durable functional restoration in the future.

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