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Emerging trends and role of biologically active metalloproteinases enzymes

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Abstract

Limb model is used to explain the mechanism of regeneration in axolotl. This model is based on classic studies demonstrating that an entire limb can be induced to form on the side of the arm when appropriate signals from nerves and connective tissues, fibroblasts are provided. The unique ability of amphibians to regenerate whole limbs demonstrates a greater capacity for regeneration and healing when compared with wound healing in mammals. Regeneration of amputated digits has been documented in

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higher-order mammals in the embryonic and neonatal stages. MMP7 also suggested to have a possible role in postinfarction angiogenesis, as demonstrated by plasminogen activation by increased secretion of urokinase-type plasminogen activator, which is known to degrade basement proteins and activate growth factors involved in angiogenesis. MMPs contribute to the digit or limb regeneration by promoting ECM degradation and the formation of the wound epidermis, which is formed by the migration of epidermal cells to the perimeter of the amputation surface and is necessary for wound closure.

INTRODUCTION

Regeneration of tissues occurs in all adult tetrapod's, the ability to regenerate complex structures such as limbs is limited inurodeles (newts and salamanders). Many of biological processes and signaling pathways that control these processes are highly conserved among all tetrapod (McCusker and Gardiner 2011). Limb Model is used to explain the mechanism of regeneration in axolotl. This model is based on classic studies demonstrating that an entire limb can be induced to form on the side of the arm when appropriate signals from nerves and connective tissue fibroblasts are provided. This model reduces the complexity associated with amputations in which considerable damage is done to limb tissues, resulting in injury responses that associated with signalling that induces regeneration (Muri et al.,2019). Additional signals from the nerve are stimulate the dermal fibroblasts to dedifferentiate and migrate toward the nerve and aggregate to form the early blastema (Pondeljak et al.,2020). Dermal cells from the opposite side of the limb are grafted to the wound site, the ectopic blastema continues to the next steps in the regeneration pathway and a new limb is formed. One important point is that regeneration-competent cells will fail to form a regenerate unless all these criteria are met. The challenge is to identify each step and determine which steps are the

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barriers to regenerative success (Han *et al.,* 2001).

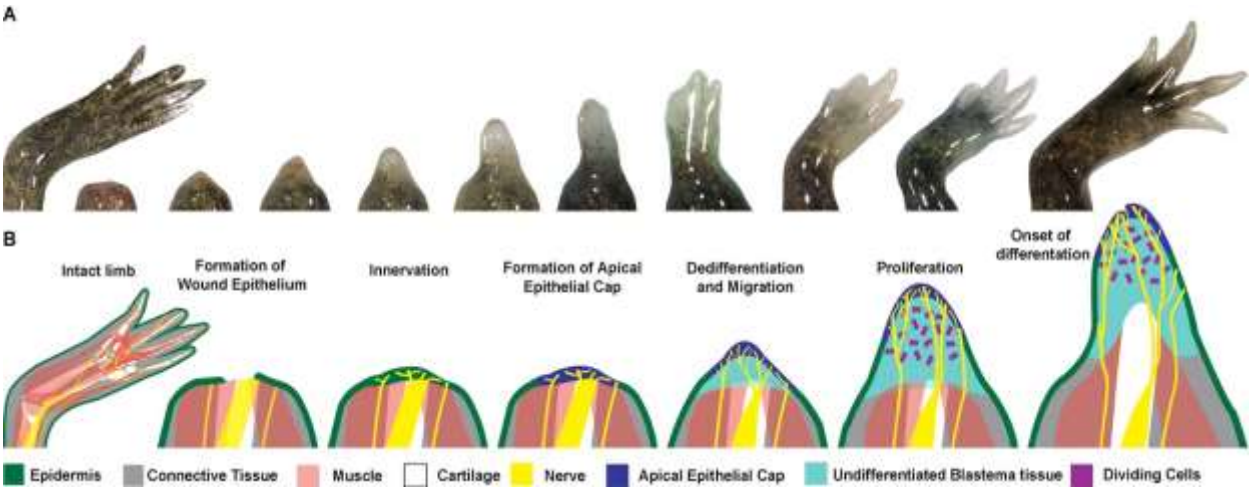

Fig 1: (A): limbs and Induction features (B) different development processes in the axolotl (*Ambystoma mexicanum*)

Mechanism of Limb regeneration

The speed of salamander limb regeneration varies among species and developmental stage but is nevertheless impressive. The best staging, based on meticulous histological series, is available for *Notophthalmus*, which completes adult limb regeneration within less than 2 months. Immune cells also populate the area and become activated, and systemic depletion of macrophages during an early, sensitive period of the regeneration event results in wound closure but permanent failure of limb regeneration (Godwin *et al.,* 2013). Underneath the wound epidermis, stump cells begin to populate the blastema. By the second week after amputation, the blastema has grown noticeably, and by the third week the initial stages of an elbow bend and a flattening hand palette can be discerned. Thus, 1 month after amputation, a salamander limb can regenerate its complex features; it then spends an additional month growing back to its original size(Davidian *et al.*,2021)..

The mechanism behind MMPs in myoblast differentiation/fusion process may be

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because these enzymes can serve to eliminate ECM and/or cell surface components that intercalate and thereby hinder the fusion between two cell membranes (Checchi *et al.*,2020). Besides the fusion itself, the preceding steps, such as migration and alignment of myoblasts, may also be affected directly by these MMPs. Cultured embryonic cells failed to form myotubes efficiently in the absence of MMP-2 and MT1-MMP, indicating that MMP-2 and MT1-MMP are the major factors that alter myotube formation in vitro. In fact, the correlation between the expression of MMPs and TIMPs in myogenic differentiation has long been investigated.11,13 Masseter myotubes express high levels of TIMP-1 mRNA without MMP-9 activity in vitro. This situation is highly analogous to that observed in masseter muscle in vivo, where TIMP-1 protein is abundantly localized to the basal lamina and endomysium of muscle myofibres while MMP-9 protein is detected in a very low level (El Fahime *et al.,* 2000; Lewis *et al.,* 2000).

Role of MMPs in Limb regeneration of axolotl

The unique ability of amphibians to regenerate whole limbs demonstrates a greater capacity for regeneration and healing when compared with wound healing in mammals. Regeneration of amputated digits has been documented in higher-order mammals in the embryonic and neonatal stages(Godwin *et al.,* 2013). Both processes of wound healing in mammals and limb regeneration in axolotl use MMPs to guide the remodelling of damaged tissue. In the days following inflammation, MMPs play a more active part in re-epithelialization of the wound. Other studies provided evidence that MMP9 contributed to limb regeneration by digesting gelatin along with collagen I and IV, thereby removing damaged cartilage elements (Vinarsky *et al.,* 2005).

In another study based on histolysis in regeneration-competent urodele limbs carried out by acid hydrolases, MMPs, and HCl shows that The major histolytic role played

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by MMPs, and the importance of histolysis to the success of regeneration have been emphasized by the failure of blastema formation in amputated newt limbs treated with the MMP inhibitor GM6001 (Vinarsky *et al.,* 2005). MMP transcripts that have been identified in regenerating axolotl and newt limbs include the gelatinases MMP-2 and -9, and the stromelysins MMP-3/10a and b. These MMPs would thus also appear to play a significant role in histolysis of the regenerating wild-type axolotl limb(Kato *et al.,* 2003; Vinarsky *et al.,* 2005).

In another study expression of MMP-9 and -2 was compared by zymography between regeneration-competent wild-type axolotls and stage-54 Xenopus, and regeneration-deficient short-toes axolotls and Xenopus froglets (Santosh *et al.,* 2011). The results show that the Both short-toes and froglet failed to up-regulate MMPs in a pattern comparable to the wild-type axolotl, suggesting that subnormal histolysis is at least in part responsible for the poor blastema formation characteristic of both short-toes and froglet(Liu et al.,2022). MMP levels were much lower in amputated stage-54 Xenopus limb buds than in the other animals, suggesting that blastema formation in these limb buds requires much less extracellular matrix degradation than in fully differentiated limbs. TIMP expression patterns followed the same trends as the MMP's in each group of animals (Santosh *et al.,* 2011).

Through pharmacological studies, MMP activity appears necessary for tissue dedifferentiation and blastema formation in newt limb regeneration (Vinarsky *et al.,* 2005). Intriguingly, MMP1 is involved in *Drosophila* leg disc regeneration. Although MMP1 reduction did not influence blastema formation or growth, it affected the proliferation of the surrounding leg disc cells (McClure *et al.,* 2008). The regeneration of digit tips, the digit, and even the whole limb has been intensively studied in the field of regenerative

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medicine. For newts or fetal mice, the amputated limbs or digit tips were observed to fully regenerate, which does not normally occur in mammalian wound healing (Muneoka *et al.,* 2008). MMPs have been found to be up-regulated very early after digit or limb amputation and are required for this regeneration process. The healing of a wound or wound closure is the first step in digit or limb regeneration. It was proposed that MMPs contribute to the digit or limb regeneration by promoting ECM degradation and the formation of the wound epidermis, which is formed by the migration of epidermal cells to the perimeter of the amputation surface and is necessary for wound closure (Stoick-Cooper *et al.,* 2007). Although numerous studies have shown that amputation of a digit tip distal to the mid-third phalanx resulted in almost complete regeneration, amputation proximal to this region does not support regeneration without assistance from exogenous factors. Due to the numerous beneficial effects of MMP1, we proposed to investigate the effect of MMP1 treatment in improving the wound healing process and reducing scar formation, e.g. scarless healing, after digit amputation (Atkinson *et al.,* 2005).

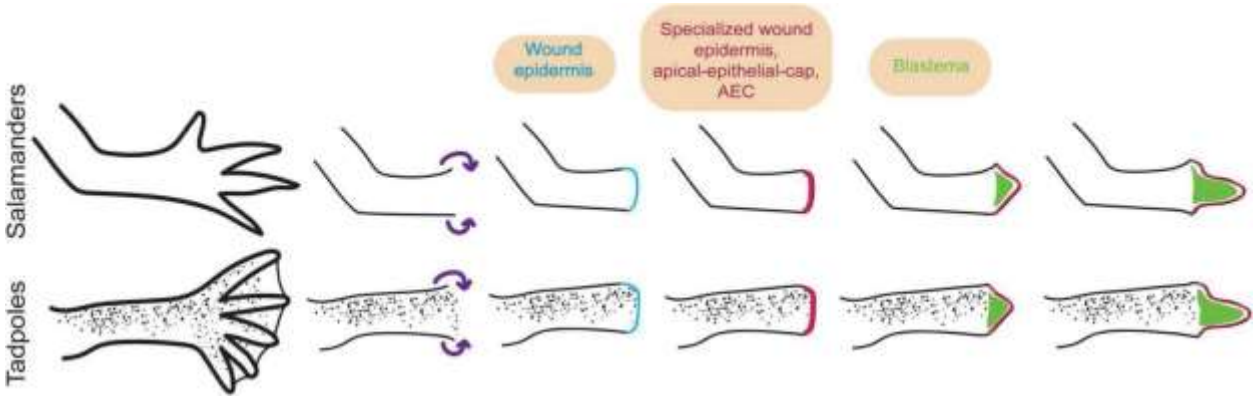

Fig 2: Shows the features and Role of Limb regeneration

Several expressed sequence tag (EST) projects for axolotls yielded important sequence

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and expression information. This type of strategy converts mRNA from expressed genes into cDNA, clones the cDNAs to create a library, and then sequences into the cDNA inserts, reading several hundreds of bases into the ends of the original transcripts. This helps the researchers to identify the function of MMPs during embryo limb regeneration. The era of transcriptomics has revolutionized gene discovery in axolotls. RNA-seq makes no presumptions about the identity of the transcripts at play except that they can be captured in the purification protocol and subjected to reverse transcription, adapter ligation, and amplification. Through a combination of microarray and RNA-seq approaches along with other approaches and extensions should provide an even more comprehensive understanding of the molecular factors and role of MMPs enabling limb regeneration. Epigenetics and Proteomic approaches together with RNA-seq studies enables to study the exact role of Matrix Metalloproteinases in the limb formation of axolotl.

Table 1: Role of various mmps in axolotl limb regeneration

Gene symbol	Gene name	Expression pattern	Proposed function	Type of study
mmp11	matrix metalloproteinase 11	Blastema-enriched	Wound healing/matrix remodeling?	RNA-seq; candidate
mmp1	matrix metalloproteinase 1	Enriched in 0–1 dpa over 2–3 dpa	Wound healing/matrix remodeling?	Microarray

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mmp3	matrix metalloproteinase 3	Mid-blastema; enriched in 0–1 dpa over 2–3 dpa	Wound healing/matrix remodeling?	Subtractive hybridization
mmp9	matrix metalloproteinase 9	Blastema (early mid), wound epidermis/heterogeneous, possibly downstream of innervation	Wound healing/matrix remodeling?	Microarray
mmp1	matrix metalloproteinase 10	Enriched in 0–1 dpa over 2–3 dpa	Wound healing/matrix remodeling	Microarray

Function of Different MMPs in Axolotl Limb Regeneration

Over the past four decades, more than 25 vertebrate MMPs have been identified and classified according to their protein structure and substrate specificity(Fu et al.,2022). These groups include collagenases (MMP1, MMP8, MMP13, and MMP18), gelatinases (MMP2 and MMP9), stromelysins (MMP3, MMP10, and MMP11), and the membrane-type MMPs (MMP14, MMP15, MMP16, MMP17, MMP24, and MMP25). Most MMPs are secreted in a latent form and must be activated by cleavage of an N-terminal propeptide domain. Activation can occur through the actions of other proteases, chaotropic agents, or organomercuric compounds such as 4-aminophenylmercuric acetate (APMA). Although MMP activity is known to increase during salamander limb regeneration. In

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salamanders, two stromelysins were identified for *N. viridescens* and designated (*NvMmp3/10a* and *NvMmp3/10b*) (Vinarsky *et al.,* 2005). These two genes were shown to have homologs in *C. pyrrhogaster* (*CpMmp3/10a and CpMmp3/10b*) (Kato *et al.,* 2003). Axolotls were shown to have three different *mmp3* that share high sequence identity to these genes (Monaghan, 2009). Another study revealed a previously uncharacterized fourth stromelysin in in the axolotl (Al Haj Baddar, 2019). Study showed axolotl putative MMP3 paralogs as *mmp3a*, *mmp3b*, *mmp3c*, and *mmp3d*. All of these axolotl paralogs cluster with Nv- and Cp MMP3/10 a and b in the phylogenetic tree, along with putative stromelysin homologs in other salamander species. Surprisingly, stromelysins from other vertebrates (human, zebrafish, chicken, and frogs) were not included within this clade, suggesting significant stromelysin sequence divergence in salamanders (Al Haj Baddar, 2019).

A novel MMP (designated as nMMPe) from *C. pyrrhogaster* that showed low sequence similarity to other vertebrate MMPs (Kato *et al.,* 2003). The cloned gene was shown to have a threonine-rich 42 amino acid insertion in the hinge domain. Another distinctive feature of this novel MMP was a proline to serine amino acid substitution in the conserved cysteine switch motif PRCG (V/N) PD. Another study identified presumptive orthologs for *nmmpe* in the axolotl, *A. maculatum*, *A. andersoni*, *N. viridescens*, and *P. waltl.* However, some of these genes did not cluster together as would be expected if they shared a common evolutionary history (Al Haj Baddar, 2019).

Two different *mmp13* transcripts are identified from Sal-Site that encode different protein-coding sequences. We denote these as *mmp13a* and *mmp13b*; the former matches sequence "*ammp13*" identified previously by Monaghan (2009) and *mmp13b* is the homolog of *C. pyrrhogaster mmp13*. Both axolotl *mmp13* sequences cluster within

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the MMP13 clade that includes MMP13 orthologs from *H. sapiens*, *G. gallus*, *X. tropicalis*, and *N. parkeri*. Two salamanders (*A. andersoni* and *B. ramose*) were found to have homologs for both MMP13 sequences, while other salamander species (*A. maculatum*, *H. chinensis*, *N. viridescens*, and *P. walti*) had only one *mmp13* homolog. This suggests that the *mmp13* duplication occurred in an ancestor of ambystomatid and plethodontid salamanders. However, most family-level reconstructions of urodeles suggest that salamanadrids are more closely related to ambystomatids. If this is true, newt species would also be expected to have two *mmp13* genes. Additional data are needed to reconstruct the evolution of *mmp13* paralogs (Al Haj Baddar, 2019).

Various studies have shown the diverse number of tasks MMPs are involved in during valve and wall remodeling (Liu et al.,2022). In one study, while MMP9 expression was increased in late scarring from MI, MMP2 was the predominant gelatinase that increased in decompensated left ventricular (LV) myocardium by cardiomyocytes for continued LV remodeling. An increase in MMP7 activity was observed in scar myocardium, suggesting involvement in proteoglycan degradation through secretion by local fibroblasts. MMP7 was also suggested to have a possible role in postinfarction angiogenesis, as demonstrated by plasminogen activation by increased secretion of urokinase-type plasminogen activator, which is known to degrade basement proteins and activate growth factors involved in angiogenesis (Zeng *et al.,* 2002).

Conclusion

MMPs or TIMPs have an integral role in a specific diseases and limb regeneration. MMP3 and MMP 13 play a significant role in regeneration of axolotl because these collagenases early prevent basal lamina formation during regeneration and ensure the communication between the wound epidermis and mesenchyme. In all axolotl *mmp3* transcripts peaked

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early after the limb amputation and genes of mmp3 expressed in basal layer of the wound epidermis and triggers the dramatic upregulation of transcripts in salamanders through the shared mechanism. Majority of axolotl mmps involved in the formation and maintenance of blastema.

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Conflict of interest

Authors declare no conflict of interest

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