Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Waqas et al - 2026

DOI: http://doi.org/10.5281/zenodo.20083373

Role, Innovative Features, And Applications of Novel Enzymes in Animal Physiology

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Pakistan Journal of Medical & Cardiological Review

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Author Details

Keywords:
Matrix metalloproteinases, limb regeneration, inhibitors, urodele amphibians

Received on 10 March, 2026
Accepted on 20 April, 2026
Published on 04 May, 2026

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Abstract

Regeneration of limb tissues and other organs in urodeles can be controlled through biological processes that are highly conserved in tetrapod and like humans that have a potential to regenerate their complex structures. They regenerate their lost appendages through different processes that involved in proliferation, differentiation and migration of proximal cells of amputation. Metalloproteinases (MMPs) are important group of proteases consist of a catalytic and peptide domains where the zinc ion binds and participates in the remolding of extracellular matrix by degrading the certain constituents. MMPs are involved in improving the potential of wound to regenerate. Level of MMPs 3, MMP 9 and MMP 13 upregulated during the amputation of limbs and shows the highest expression of these proteins for the proteolytic activity of limb. Matrix metalloproteinases are required or essential for the limb regeneration of axolotls and prevent the formation of scars during regeneration process. This review article focused on the structure, regulation of MMPs and TIMPs, mechanism of action and roles of matrix metalloproteinases in limb regeneration in urodele amphibians (Axolotls).

INTRODUCTION

Regeneration is a part of life and most of organisms have ability to replace old and damaged cells. Regeneration is essential not only for tissue homeostasis, but also for the prolonged survival of the organism. However, the ability to regenerate more complex structures, such as limbs, is greatly variable among tetrapod species. It has been well-documented that these abilities to replace damage tissues during homeostasis and to heal acute injuries are negatively affected by aging in most organisms(Muri *et al.*,2019). All tetrapods have ability to regenerate their complex structures such as organs or limbs as embryos, but many of these species, including mammals, lose this capacity as they develop into adult organisms. While others, including Urodele amphibians (salamanders and newts), are capable of regenerating throughout adulthood. Limb regeneration in urodele amphibians such as the axolotl (*Ambystoma mexicanum*) occurs by the formation of a blastema, a mass of mesenchymal stem-like cells that resembles the early embryonic limb bud (Tanaka, 2003; Stocum and Rao, 2010).

The phenomenon of limb regeneration is important in most living organisms. Blastema formation is formed through degradation of extracellular matrix (ECM) to release the dermal fibroblasts (Davidian *et al.,*2022). Limb amputation, epithelial cells proximal to the amputation plane converted to a migratory form and cover the wound completely within 24 hours, while internal limb cells start to dedifferentiate to form a mass of proliferating progenitor cells known as the regeneration blastema. The newly-formed wound epithelium (WE) is required for the growth of the regenerating salamander limb and its later derivative, the apical epithelial cap (AEC)even promote the supernumerary limb formation when transplanted to a more proximal region of an existing regeneration blastema (Pulawska et al.,2021).

Matrix metalloproteinases (MMPs) belong to a superfamily of enzymes known as metazincins, which encompass several other endopeptidases including serralysins, asatacins, adamalysins, leishmanolysins, snapalysins and pappalysins (Huxley-Jones *et al.,* 2007). Furthermore, these enzymes have similar structures, including a signalling peptide, a propeptide domain, a catalytic domain where the zinc ion binding site resides and a hinge region that binds to the C-terminal hemoplexin domain (Visse and Nagase, 2003). A majority of MMPs are secreted in a latent form known as a pro-MMP and can only become active when the bond between the free thiol of a conserved cysteine residue on the propeptide domain and the zinc ion on the catalytic domain is broken or through complete cleavage of the propeptide domain through the use of other MMPs (Ra and Parks 2007).

All MMPs are known for their involvement in several biological processes(Dwaraka et al;,2019). Generally, these enzymes played important role in in embryonic development and are almost undetectable in normal adult resting tissues; however, they become clearly activated when perturbed through injury, disease and during pregnancy (Choi *et al.,* 2004). While some MMPs are known primarily for their ability to degrade certain components of the extracellular matrix (ECM), they are not solely limited to this physiological task (Parks *et al.,* 2004). When stimulated, MMPs interact with various cytokines and chemokines to become engaged in different roles such as cell proliferation, migration, differentiation, apoptosis and angiogenesis(Zeng *et al.,* 2009)

Limb regeneration follows a different course for amphibians, yet MMP activity is still present. Almost instantly after a limb amputation of a newt, MMP activity becomes highly elevated in order to rapidly produce an apical epithelial cap to cover the stump, minimizing additional tissue damage, contamination or inflammatory response (Yokoyama, 2008). Sequence homology between MMPs MMP3/10a and MMP3/10b expressed in newts and humans only for regenerating limbs and not in normal tissue. Both MMPs aided in tissue remodelling by digesting gelatin, casein and collagen I and IV. MMP9 contributed to limb regeneration by digesting gelatin along with collagen I and IV, thereby removing damaged cartilage elements (Vinarsky *et al.,* 2005).

This review article focussed on the structure and regulation of MMPs and TIMPs also mechanism of action and roles of the mechanism of limb regeneration in urodele amphibians i.e. axolotls and how matrix metalloproteinase effects the process of limb regeneration in axolotls.

Matrix Metalloproteinases (MMPs)

MMPs belong to a superfamily of enzymes known as metazincins, which encompass a number of other endopeptidases including serralysins, asatacins, adamalysins, leishmanolysins, snapalysins and pappalysins (Huxley-Jones *et al.,* 2007). Currently, there are 23 knowns human MMPs, with other species having slightly variable structures, all of which share similar characteristics (e.g., a zinc ion binding site) and are inhibited by tissue inhibitors of metalloproteinases (TIMPs) as shown in **Table 1**.

Table 1. Classification of MMPs on the basis of their substrate specificity

Subgroups	MMP	Name	Substrate
Collagenases	MMP-1	Collagenase-1	Col Ⅰ, Ⅱ, Ⅲ, Ⅶ, Ⅷ, Ⅹ, gelatin.
	MMP-8	Collagenase-2	Col Ⅰ, Ⅱ, Ⅲ, Ⅶ, Ⅷ, Ⅹ, gelatin, aggrecan.
	MMP-13	Collagenase-3	Col Ⅰ, Ⅱ, Ⅲ, Ⅳ, Ⅸ, Ⅹ, ⅪⅤ, gelatin.
Gelatinases	MMP-2	Gelatinase-A	Gelatin, Col Ⅰ, Ⅱ, Ⅲ, Ⅳ, Ⅶ, Ⅹ

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	MMP-9	Gelatinase-B	Gelatin, Col Ⅳ, Ⅴ
Stromelysins	MMP-3	Stromelysin-1	Col Ⅱ, Ⅳ, Ⅸ, Ⅹ, Ⅺ, gelatin.
	MMP-10	Stromelysin-2	Col Ⅳ, laminin, fibronectin, elastin.
	MMP-11	Stromelysin-3	Col Ⅳ, Fibronectin, liminin, aggrecan.
Matrilysins	MMP-7	Matrilysin-1	Fibronectin, liminin, Col Ⅳ, gelatin.
	MMP-26	Matrilysin-2	Fibrinogen, fibronectin, gelatin.
MT-MMP	MMP-14	MT1-MMP	Gelatin, fibronectin, laminin.
	MMP-15	MT2-MMP	Gelatin, fibronectin, laminin.
	MMP-16	MT3-MMP	Gelatin, fibronectin, laminin.
	MMP-17	MT4-MMP	Fibrinogen, fibrin.
	MMP-24	MT5-MMP	Gelatin, fibronectin, laminin.
	MMP-25	MT6-MMP	Gelatin.
	MMP-12	Macrophage metallo-elastase	Elastin, fibronectin, Col Ⅳ.

Others	MMP-19	-	Aggrecan, elastin, fibrillin, Col Ⅳ, gelatin.
	MMP-20	Enamelysin	Aggrecan.
	MMP-21	XMMP	Aggrecan.
	MMP-23	-	Gelatin, casein, fibronectin.
	MMP-27	CMMP	Unknown.
	MMP-28	Epilysin	Unknown.

Complementary to MMPs, four inhibitors known as TIMPs as shown in Table 2, which serve the purpose of inhibiting MMPs, in addition to closely related members of the adamalysin group, a disintegrin and a metalloproteinase (ADAMs) (Baker *et al.,* 2002). Like most MMPs, TIMPs are secretory proteins that regulate the degradation of ECM constituents and tissue remodelling through interaction with MMPs. These inhibitors limit the extent of MMP participation in the regenerative process by restricting cellular functions such as proliferation and migration (Stetler-Stevenson, 2008). The typical shape of TIMPs is wedge-like, containing both an N- and C-terminal domains with a molecular weight ranging from 21 to 29 kDa (Somerville *et al.,* 2003). Both terminals consist of six conserved cysteine residues forming three disulfide bonds; however, only the N terminal is responsible for inhibiting MMP activity (Visse and Nagase 2003).

Table 2: Biological matrix metallo-proteinase inhibitors

Inhibitor	Expressions	Tissue Distribution	Location	Function
TIMP-1	Inducible	Wide	Secreted	Inhibition of MMPs, except MMP-14, 15, 16, 19, 24 and ADAM-10.
TIMP-2	Constituted	Wide	Secreted	Inhibition of MMPs, ADAM-12 and activates MMP-2, 3, 13.
TIMP-3	Inducible	Wide	Extracellular matrix	Inhibition of all MMPs, ADAM-10, 12, 17 and ADAMST-2, 4, 5.
TIMP-4	Restricted and highly regulated	Brain, Heart, Kidney, Ovary, Testes, Colon, Pancreas, Fat	Secreted	Inhibits all MMPs.

Structure of MMPs:

MMPs are well characterized in structure having catalytic properties to degrade extracellular matrix. In response to inflammation and cellular activation, MMPs are synthesized by a variety of cell types including endothelial and epithelial cells, leukocytes, neural cells, and hepatocytes. MMPs play a significant role in immunological functions by including extracellular matrix components (ECM) in leukocyte migration, modulating chemokine and cytokine activity through both their inactivation and activation (soluble factors) as well as by defensive activation (Parks *et al.,* 2004). MMPs are found to be involved in various physiological processes such as embryogenesis, reproduction, angiogenesis and in pathological conditions such as tumor invasion by facilitating degradation of basement membrane and inflammation (Webster and Crowe, 2006). MMPs are divided into three domains based on their structure (Fig. 2). An amino-terminal pro-peptide region, 2) An amino-terminal catalytic domain (which contains the zinc-binding motif) and 3) A carboxyl-terminal domain.

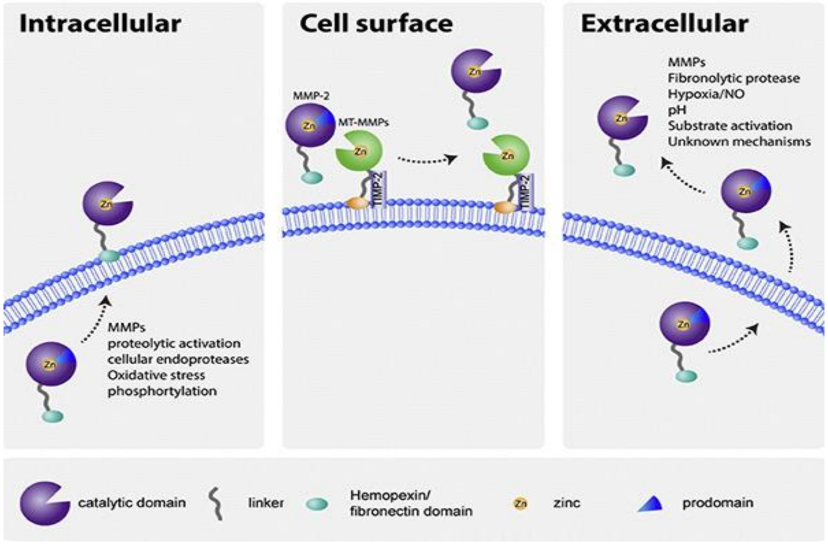

Fig 1: Shows the features of MMPs on the basis of their substrate specificity

MMPs are very similar to members of the hemopexin family and are involved in binding with substrate ECM for different MMPs(Davidian et al.,2022). The carboxyl-terminal end of hemopexinlike domain is a four-bladed β-propeller structure which is found in all MMPs except MMP7 and MMP26.

A hinge region, which is long in other MMPs and short in collagenases are used to connect the carboxyl and amino-terminal domains. Before the pro-peptide region, a short signal sequence (pre-domain) lies at the end of a protein having an amino group, which is clipped off as new MMPs are synthesized. Six MTMMPs are membrane proteins. MT4-

MMP (MMP17) and MT6-MMP (MMP25) are glycosylphosphatidylinositol (GPI)-anchored MMPs, whereas the other MT-MMPs are transmembrane proteins (Webster and Crowe, 2006).

Regulation of MMPs and TIMPs:

MMPs and TIMPs are potent proteolytic enzymes that are capable of widespread destruction. Most of the MMPs are not constitutively expressed but are transcribed after cell activation. MMPs are essential regulators of tissue homoeostasis and immunity in the network of multidirectional communication within tissues and cells (Checchi et al.,2020). Since, uncontrolled MMP activity can easily become destructive and lead the breakdown of homoeostasis, their activity should be tightly regulated(Pulawska et al.,2021).

MMPs activity is regulated at four different levels: 1) gene expression with transcriptional and post-transcriptional regulation; 2) extracellular localization and tissue or cell type of MMP release, termed compartmentalization; 3) pro-enzyme activation by removal of the pro-domain; and 4) inhibition by specific inhibitors, i.e. tissue inhibitors of matrix metalloproteinases (TIMPs), and by non-specific proteinase inhibitors, e.g. a2-macroglobulin. In active form, MMPs can modulate the global proteolytic potential in the extracellular milieu through zymogen (MMP pro-form) activation and inhibitor degradation or inactivation of other proteases (Overall and López-Otín, 2002; Ra and Parks, 2007).

DOI: http://doi.org/10.5281/zenodo.20083373

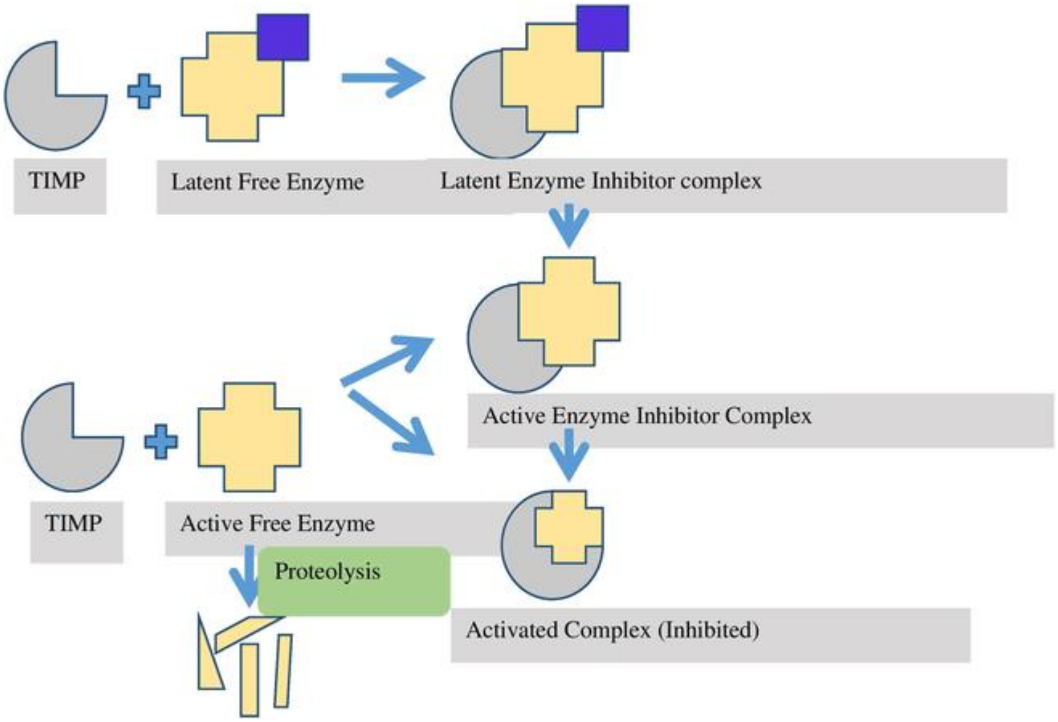

Figure 2: Shows the mechanism of Regulation of MMPs and TMPs

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 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.

Acknowledgments

All authors have contributed, finding related articles and material.

Conflict of interest

Authors declare no conflict of interest

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