INTRODUCTION

> *"Nature gives back to the animal precisely and only that which it has lost, and she gives back to it all that it has lost"*
> *(René-Antoine Ferchault de Réaumur, 1712)*

Regeneration can be defined as the natural ability of living organisms to replace worn out parts, repair or renew damaged or lost parts of the body, or to reconstitute the whole body from a small fragment during the post embryonic life of an organism. Regeneration is thus also a developmental process that involves growth, morphogenesis and differentiation. Nonetheless, true tissue regeneration or complete regeneration refers to the replacement of lost or damaged tissue with an 'exact' copy, such that both morphology and functionality are completely restored (You and Han, 2014).

All organisms have evolved tactics to conserve their function and form throughout adult life. Many differentiated cell types are short-lived and must be constantly replaced. Noticeable examples of such homeostatic replacement are found in the vertebrate blood lineage, gut epithelium, and skin (Stoick-cooper *et al*., 2007a). Likewise, many organisms freely part with substantial parts of their body and replace them, in many cases repeatedly throughout life. Examples include the shedding cycles of the crustacean exoskeleton and snake skin, the annual replacement of bird feathers, the dramatic loss and regrowth of deer antlers, and the renewal of the endometrium in mammals (Stoick-cooper *et al*., 2007a). In addition to these physiological phenomena of reconstruction, all animals have means of dealing with damage due to injury or disease. Curing of epidermal wounds is an effectual process of repair in most organisms, but the capability to recover from damage to other organs or structures show a discrepancy widely in different organisms.

HISTORY OF REGENERATION

History of regeneration is very rich and it actually dates even back to the times of antiquity with the ancient Greeks and the beginning of civilization, or at least, when civilization began to record its activities. In fact, it actually made it into the regular traditions of ancient cultures. The regeneration of organs and appendages is a theme found in the ancient Greek

mythologies of the Hydra and of Prometheus, recounted by Homer and Hesiod (Dinsmore, 1998). The interesting thing is that the same culture that produced this mythology actually was one of the first cultures to record or ponder about the natural events of regeneration. The interesting thing about hydra is that it's a very old, basal metazoan. These animals can regenerate and therefore makes us think that regeneration may be just as old as multicellular life because we can find a fossil record for Cnidarians that actually dates back all the way to the Vendian period which is about 650-540 million years ago. The first likely citation for a type of regeneration was written by Empedocles, who lived from 490-430 BCE, where he mentioned about the existence of necks that were lacking domains or heads that were going to actually grow these heads again. These writings, of course, passed on from generation to generation in ancient Greek. Even Aristotle was aware and he has mentioned a series of curiosities, such as lizard tail regeneration, which is very likely he did not see, but actually heard from second or third hand, and nonetheless, included in his literature. Hence, already the Greeks knew that some animals were capable of restoring lost and missing body parts. Hundreds of years went by from the time the Greeks wrote all this.

At the end or at the middle of the 17th century the French began to develop an interest in natural history and began to record their activities for mankind. One of the first known empirical examinations of regeneration a thousand years later from the initial recordings of this by Aristotle, was carried out by Melchisedech Thévenot. He set up some type of diorama of sorts where he would amputate the tail of the lizard, and then allowed the visitors of the French Academy of Science to actually follow the regeneration of the lizard day after day after day which was recorded, it took place in 1686. At about the same time, a few years later, the French scientist, René Antoine de Réamur, was one of the first individuals to publish an observation of regeneration. He did this in 1712, the beginning of the 18th century. In 1712 he wrote a manuscript that describes what he thought was the capacity of crabs and lobsters and so forth, to regenerate their limbs. Abraham Trembley in 1744 essentially worked with this organism, which we know today as hydra micropolyps. These organisms even today, have survived as a model system to study regeneration. The 18th century work of Italian priest Lazzaro Spallanzani reported the regenerative properties of a number of animals in detail and demonstrated that regeneration actually was capable of taking place in even higher organisms, or more complex organisms, like vertebrates. Since then, this field has always fascinated many researchers. Studies on limb development and regeneration in the latter part of the 19th and the early part of the 20th centuries made major

contributions to the understanding of development. In the early 20th century, T.H. Morgan scientifically studied the ability of planaria to regenerate even after being cut into several pieces (Morgan, 1901). Later studies have continued to focus interest on animals with high regenerative capacities (Birnbaum and Sanchez-Alvarado, 2008). A summary of contribution by scientists and their work on regeneration is given in the following table (Table 1).

Scientist	**Regeneration study**
Abraham Trembley (1744)	First scientific discovery of regeneration in hydra
Lazzaro Spallanzani (1765)	Announcement of regeneration of tails and limbs in Salamanders; in "Prodromo"
Charles Bonnet (1762)	Reporting several regeneration systems in Salamanders (including lens regeneration)
Tweedy John Todd (1823)	Discovery of "Neurotrophic effects" on the limb regeneration
Gustav Wolff (1894)	Announcement of the regeneration of the lens from dorsal Iris in Newts
Thomas Hunt Morgan (1901)	Coined the term morphallaxis and epimorphosis.
Elmer Grinshaw Butler (1933)	Establishment of de-differentiated mesoderm as a source of regeneration Blastema
Goro Eguchi and Okada (1973)	Demonstration of cell transdifferentiation in *in vitro* systems
Niazi and Saxena (1978)	Induction of abnormal limb regeneration by Vitamin A in anuran tadpoles
Susan Bryant and others (1981)	Interpretation of the pattern formation in the regeneration in terms of positional information
Priyambada Mohanti-Hejmadi *et al*., (1992)	Induction of Heteromorphic regeneration by Vitamin A and retinoids in tadpoles
Jeromy Brockes *et al.*, (2007)	Newt Anterior Grade protein secreted by the nerve sheath drives the regeneration of the limb from the stump

Table 1: List of scientists and their particular contribution to the regeneration field (adapted from Okada, 1996)

REGENERATION IN DIFFERENT GROUPS OF ANIMALS

Repair and regeneration are universal phenomena in the biological world, but the capacity for regeneration varies considerably among the taxonomical hierarchy. Arthropods are known to regenerate appendages following loss or autotomy (Bliss and Mantel, 2012). Other invertebrates, such as planarians or annelid worms, can regrow all missing body parts when cut into small pieces, while similarly complex nematodes do not regenerate at all (Slack *et al*, 2007). Likewise, some vertebrates, such as urodele amphibians (newts and salamanders) are the champions of vertebrate regeneration. They can replace lost limbs, tails, lens, retina, lower jaw and several internal organs and repair skeletal muscle, peripheral nervous system and CNS, while anuran amphibians have significantly lower regenerative capability. It thus appears that organism complexity is a rather poor predictor for regenerative capacity. Regeneration can be considered a "pristine" quality of all tissues and structures and not a specific evolutionary adaptation of a few organisms and organs (Stoick-Cooper *et al*., 2007a) Fish can also regrow amputated hearts. Certain lizards can regenerate their tails after autotomy or amputation. Mammals (including humans) can repair damage to skeletal muscle and peripheral nervous system and can recover from damage to the liver, however lack the ability of amphibians. Moreover, for mammals, the situation is seemingly different. In most instances, mammals are capable only of incomplete regeneration. Although the function of the organ may be recovered, the structure will not be restored after part of the tissue has been damaged within a specific organ.

TYPES OF REGENERATION

The process of regeneration is a complex phenomenon, possessing a number of distinct phases (Abdel-Karim *et al*., 1990, Cadinouche *et al*., 1999 and Gardiner *et al*., 2002). Therefore, on the basis of cellular mechanisms, endogenous regeneration can be divided into two broad categories: Morphallaxis and Epimorphosis. Both necessitate the participation of stem cells or other progenitor cells. During **morphallaxis**, the remaining undifferentiated cells simply migrate to the site and differentiate into the specialized cells, with little cellular proliferation. **Epimorphosis**, however, requires pre-existing stem cells or dedifferentiation-generated progenitor cells to proliferate, differentiate, and finally replace the lost cells. The process by which the hydrozoan cnidarian hydra regenerates a severed tentacle illustrates morphallaxis. The urodele amphibians, in contrast, uniquely use a cellular dedifferentiation mechanism to regenerate a variety of tissues. In fact, the incapability of mammals in this

respect makes the field of regeneration biology attention-grabbing, as an understanding of the mechanisms of regeneration in vertebrates might pave way for enhancing regenerative ability in human.

The other types of regeneration are compensatory growth and tissue regeneration. The **Compensatory growth** is a type of regenerative growth that can take place in a number of organs after the organs are either damaged, removed, or ceased to function (Widamaier *et al.,* 2006). Additionally, increased functional demand can also stimulate this growth in tissues and organs (Goss, 1965). The growth can be a result of increased cell size (compensatory hypertrophy) or an increase in cell division (compensatory hyperplasia) or both. For instance, if one kidney is surgically removed, the cells of other kidney divide at an increased rate. Ultimately, the remaining kidney can grow until its mass approaches the combined mass of two kidneys. **Tissue regeneration** is a repair of local, restricted damage to an organ mainly via restoration of only one cell type (e.g., skeletal muscle).

All these regenerative processes comprise phenomena of very different complexities. Compensatory growth and tissue regeneration are the less complicated processes than regrowth of any appendage containing many different cell types that are organized into tissues and patterned along different axes in the organism. Yet, all regenerative processes need to be tightly regulated and involve communication between different cell types through the activation of a multitude of signaling molecules.

The reactivation of a signaling molecule, which is known to regulate polarity specification in early embryonic development, during post-embryonic development, is yet to be investigated in detail in a vertebrate model of regeneration. One such signaling molecule, suspected to be playing a pivotal role during all the stages of regeneration, is bone morphogenetic protein (BMP). Currently there are insufficient reports which suggest the involvement of BMP in regeneration, mainly the one focusing on blastema stage and differentiation stage in fish fin regeneration (Crotwell *et al*., 2004). Hence, the present study investigates the temporal expression pattern of BMP during epimorphic regeneration in teleost fish *Poecilia latipinna,* since it offers various avenues of development *viz.,* re-epithelialization, proliferation, angiogenesis, chondrogenesis, apoptosis and pattern formation, that possibly been regulated through a tightly guarded gradient of BMP in time and space. Therefore, it will be of great interest to know how BMP regulates various events

during regeneration. This study potentially can shed light on yet undetermined question of fundamental importance i.e. what role does BMP signaling, if any, play in epimorphic regeneration? Moreover, exploring the molecular processes in teleost model will contribute to the better understanding of BMPs potential in the processes of regeneration.

EPIMORPHIC REGENERATION IN TELEOST FIN AND INDUCING FACTORS

Epimorphic regeneration occurs through the establishment of balanced growth state in which the blastema gives rise to all mesenchymal cells, whereas definite areas of epidermis proliferate leading to its extension, thus allowing the enlargement of the whole structure (Santos-Ruiz *et al*., 2002). This type of regeneration involves specific mechanisms that temporally and spatially regulate cell proliferation. All these involve an exact and controlled regulation of signaling molecules. In general, all the vertebrates follow common steps during the process of epimorphic regeneration. Investigational studies in several regeneration models have led to identify the major factors regulating epimorphosis during its different stages. A brief outline of these factors along with different stages is given below.

Wound Healing

After an ablation, within the first 1-3hour post amputation (hpa), cells migrate to cover the wound by forming a thin epithelial layer - wound epithelium (WE) (Figure 1). It is known that this process occurs without the proliferation of cells and does not require a blood supply (Santamaria *et al*., 1996; Poleo *et al.,* 2001; Nechiporuk and Keating, 2002; Santos-Ruiz *et al*., 2002; Bayliss *et al*., 2006) however, little is known about the molecular signals that initiate this process. Healing continues until 18-36hpa, and during this time, it is known that several signaling molecules are upregulated. It has been documented that both fgf20a and wnt10a are expressed within the first 6hpa (Whitehead *et al*., 2005; Stoick-Cooper *et al*., 2007b). Essentially, loss-of-function studies for both fgf20a and Wnt/β-catenin signaling show that, although cells migrate to cover the wound in both cases, an abnormal and incorrectly specified wound epithelium forms (Whitehead *et al*., 2005; Kawakami *et al*., 2006; Stoick-Cooper *et al*., 2007b). Healing of blood vessels happen during this time, and regenerative angiogenesis begins, the latter requiring vascular endothelial growth factor (VEGF) signaling (Huang *et al*., 2003; Bayliss *et al*., 2006).

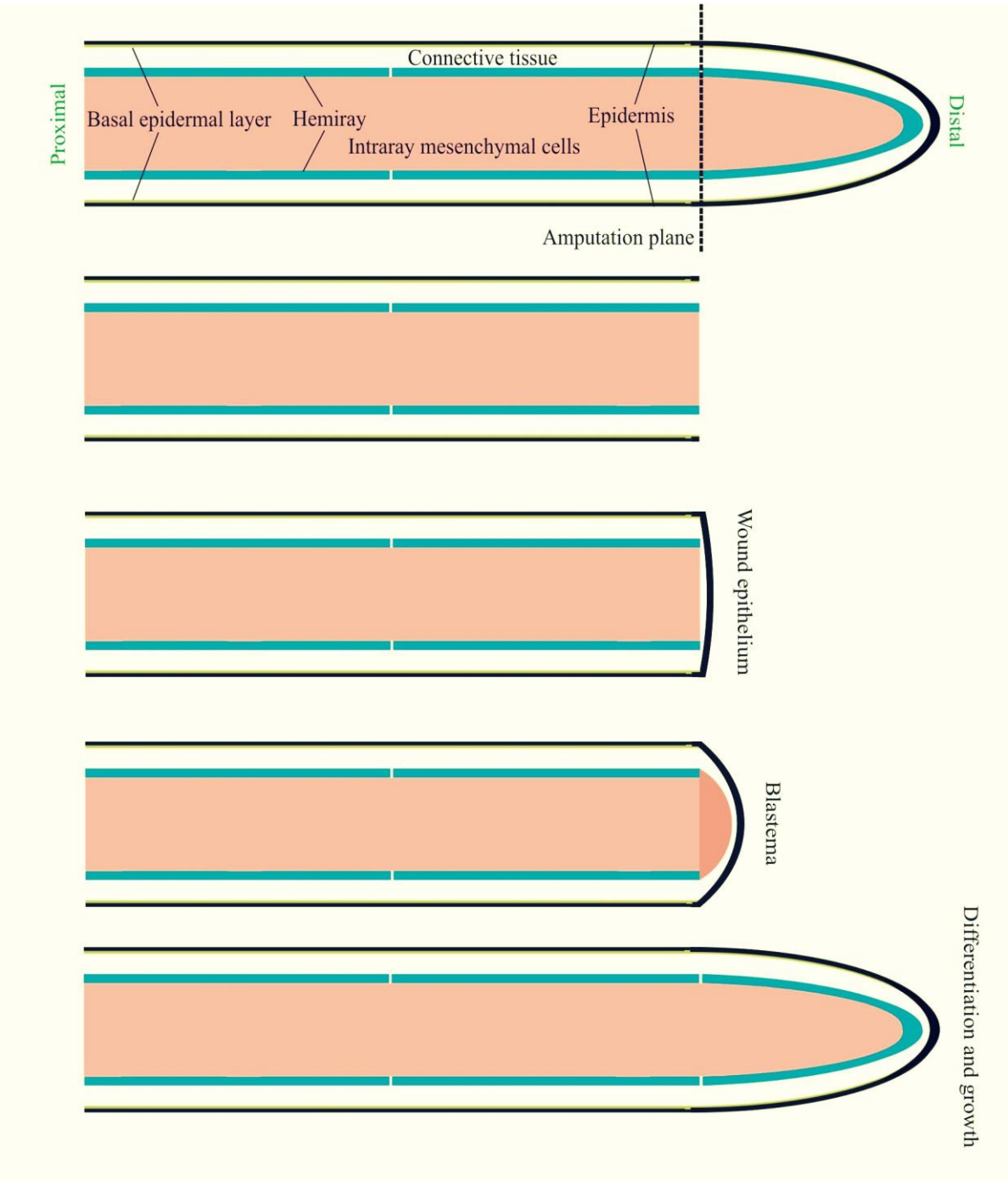

Figure 1. Stages of *Poecilia latipinna* tail fin regeneration. After amputation the epidermal cells from the edges of the wound migrate and spread over the exposed surface to form wound epithelium (within 24hpa). A few hours later, cells accumulated under the epidermis, near the site of damage dedifferentiate to form a mass, which populate the tissue distal to amputation plane forming blastema (60-72hpa). Next the dedifferentiated cells re-differentiate into different cell types. After differentiation stage balance of cell population, progenitor maintenance and re-differentiation occurs (72-120hpa) and the regenerated fin increases and attains the size of original fin (10-12dpa)

Formation of Blastema

In the succeeding phase of regeneration, a blastema gets formed that is supposed to be comprising of progenitor cells; though, it is not known where these progenitor cells come from. A blastema can easily be recognized as a white region formed on the cut surface of the body (Agata *et al*., 2007). It is possible that either mature fin cells dedifferentiate or that quiescent stem cells are activated to form this pluripotent mass. It appears likely that dedifferentiation plays the major contributing role to the formation of the blastema, however, since bromodeoxyuridine (BrdU) pulse-chase experiments have failed to reveal pre-existing slow cycling stem cells that could contribute to the formation of the blastema, there could be other sources as well (Nechiporuk and Keating, 2002). Additionally, it is also known that both Wnt/β-catenin and FGF signaling pathways are required for blastema formation because inhibition of either pathways, beginning after wound healing has taken place, results in failure to express msx genes and absence of a blastema, causing complete regeneration failure (Poss *et al*., 2000b; Stoick-Cooper *et al*., 2007a). Interestingly, there are also a couple of factors known to inhibit blastema formation when overexpressed, including Wnt5b and the chemokine, stromal cell-derived factor (SDF-1) (Dufourcq and Vriz, 2006; Stoick-Cooper *et al*., 2007b). These molecules may play a role in tightly regulating blastema formation and proliferation so that an inappropriately sized or overgrown blastema does not form.

Regenerative Outgrowth and Positional Memory

The key to regeneration is in cell differentiation. Between 2 and 4-day post amputation (dpa), the regenerating fin transitions into the outgrowth phase. The cell cycle speeds up during blastema formation to only one hour during outgrowth (Nechiporuk and Keating, 2002), and the fin begins to rapidly grow back. Blastemal cells differentiate into scleroblasts, which begin to secrete the matrix that will form the new bones, the lepidotrichia (Geraudie and Singer, 1992; Santamaria *et al*., 1992; Becerra *et al*., 1996; Mari-Beffa *et al*., 1996). The common suspects, Wnt/β-catenin signaling and FGF signaling, both are still active and important for the outgrowth phase of fin regeneration. These are required for expression of Shh, which becomes an important player during this time (Laforest *et al*., 1998; Poss *et al*., 2000a; Stoick-Cooper *et al*., 2007b). Shh begins to be expressed in cells of the basal layer of the epidermis, immediately adjacent to the newly forming dermal bone structures of the fin rays (Laforest *et al*., 1998). The expression of chordin, an inhibitor of BMP signaling, can block these effects, and the Shh signaling

inhibitor cyclopamine causes an inhibition of fin outgrowth, implicating both Shh and BMP signaling in the proliferation and/or differentiation of scleroblasts (Quint *et al*., 2002).

Recent studies further describe role of BMP signaling as being required for both blastema cell proliferation as well as bone matrix deposition (Smith *et al*., 2006). Moreover, Smith *et al* (2006) describe differential expression of sox9a/b, and a study by Avaron *et al.* (2006) shows expression of Indian hedgehog (Ihh), all known to be expressed by cartilage-forming cells, during fin regeneration. Axial and/or appendicular skeletal elements are formed via endochondral ossification while fin rays are described as dermal bones which are made via intramembranous ossification (Haas, 1962; Geraudie and Landis, 1982; Hall and Miyake, 2000). The presence of cartilage markers indicates that the dermal fin ray represents an intermediate phenotype between cartilage and bone (Avaron *et al*., 2006). However, Smith and coworkers (2006) opined that sox9a expression depends on intact BMP signaling whereas sox9b does not (Smith *et al*., 2006). Lastly, retinoic acid (RA) receptors are expressed during fin regeneration, and exogenous RA causes bifurcating bones to fuse together, suggesting a role for RA signaling in bone patterning during outgrowth (White *et al*., 1994).

Similar to regenerating limbs, amputated fins display "positional memory." That is, they grow back to the correct length, no matter where on the proximo-distal axis they were amputated, and fins amputated more proximally grow back faster than do fins amputated more distally (Akimenko *et al*., 1995; Lee *et al*., 2005).

It may be through msxb that positional memory is conferred, considering that the level of expression of msxb depends on where along the proximodistal axis the fin is amputated, with cells of the rapidly proliferating proximal blastema expressing higher levels than the cells of the less rapidly proliferating distal blastema (Akimenko *et al*., 1995; Lee *et al*., 2005).

BONE MORPHOGENETIC PROTEINS

From the time of Hippocrates, it has been known that bone has considerable potential for regeneration and repair. Nicholas Senn, a surgeon at Rush Medical College in Chicago, described the utility of antiseptic decalcified bone implants in the treatment of osteomyelitis and certain bone deformities (Senn, 1889). Pierre Lacroix proposed that there might be a

hypothetical substance, osteogenin, that might initiate bone growth (Lacroix, 1945). Nevertheless, BMPs were first identified by Dr. Marshall R. Urist, as an activity that promotes ectopic bone formation once implanted in soft tissues (Urist, 1965). Since then more than 40 types of BMPs have been described (Matthews, 2005). Bone morphogenetic proteins are a group of growth factors also known as cytokines and as metabologens (Reddi and Reddi, 2009). Several BMPs are also named cartilage-derived morphogenetic proteins (CDMPs), while others are referred to as growth differentiation factors (GDFs). BMPs are phylogenetically conserved, multi-functional secreted molecules that belong to the transforming growth factor beta (TGF-β) superfamily. The BMPs/GDFs are 30-38 kDa homodimers that are synthesized as prepropeptides of approximately 400-525 amino acids (Wozney, 1989; Hogan, 1996a; Yamashita *et al*., 1996)

BMP Receptors (BMPRs) and Signal Transduction

BMPs interact with specific receptors on the cell surface, referred to as bone morphogenetic protein receptors (BMPRs). BMPs signal through a coordinated assembly of two types of transmembrane serine/threonine kinase receptors, heteromeric complex composed of two types namely I and II. After ligand binding, the type II receptor phosphorylates and activates the type I receptor (Figure 2). Three subtypes of type I receptors have been shown to bind BMP ligands, type IA and IB BMP receptors (BMPR-IA or ALK-3 and BMPR-IB or ALK-6) and type IA activin receptor (ActR-IA or ALK-2) (Wan and Cao, 2005).

Signal transduction studies have revealed that Smad1, 5 and 8 are the immediate downstream molecules of BMP receptors, they then form a complex with co-Smad, Smad4. Later Smad1/5/8 complex translocate to the cell nucleus and function as transcription factors (Zwijsen *et al*., 2003) and plays a central role in BMP signal transduction. Phosphorylation of these specific Smads results in various cellular effects, including growth regulation and differentiation. Signaling via BMP receptors may also activate other pathways, including mitogen activated protein kinase (MAPK).

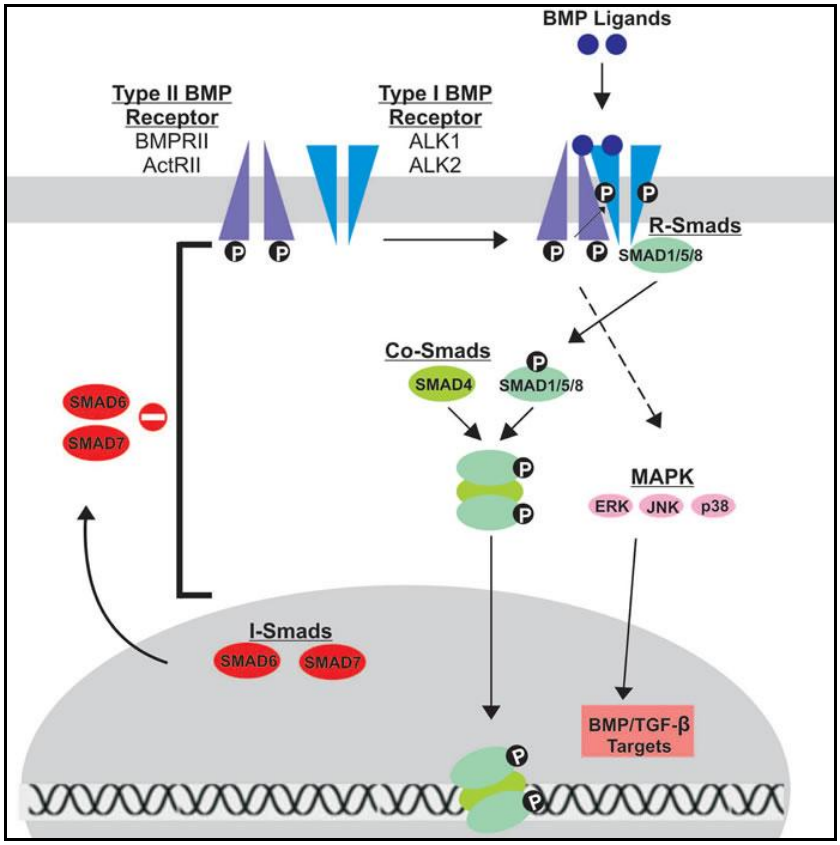

Figure. 2. BMP signal transduction (from Beederman *et al*., 2013)

Biological Activity of BMP Signaling

Originally BMPs were discovered by their ability to induce the formation of bone and cartilage. The roles of BMPs in embryonic development, embryonic patterning, early skeletal formation and cellular functions in postnatal and adult animals have been extensively studied in recent years. As such, disruption of BMP signaling can affect the body plan of the developing embryo. For example, BMP-4 and its inhibitors noggin and chordin help regulate polarity of the embryo (i.e. back to front patterning). Specifically BMP-4 and its inhibitors play a major role in neurulation and the development of the neural plate. BMP-4 signals ectoderm cells to develop into skin cells, but the secretion of inhibitors by the underlying mesoderm blocks the action of BMP-4 to allow the ectoderm to continue on its normal course of neural cell development (Wan and Cao, 2005; Miyazono *et al*., 2010).

BMPs have wide-ranging functions including dorso-ventral patterning, haematopoiesis, vascular development and homeostasis (Miyazono *et al*., 2010). They are involved in cascades of morphogenesis. BMPs have been shown to be critical for heart, kidney,

vasculature, neuron and skin development. BMPs exert an important role in skeletal development, adult bone homeostasis and fracture healing, and have demonstrated clinical utility for bone regeneration (Tsuji *et al*., 2006). A variety of model systems both *in vitro* and *in vivo* have shown the importance of TGF-β superfamily members in these processes. For example, bone morphogenetic protein-4 induces human embryonic stem cells to form a capillary like network of cells when grown on matrigel, and BMP-2 increases tumour vascularisation in mice (Langenfeld and Langenfeld, 2004; Boyd *et al*., 2007). Mice deficient in components of the BMP signal transduction pathway die *in utero* due to gastrulation defects and to a failure of vascular differentiation (Moser and Patterson, 2005). Moreover, observations from transgenic and knockout mice and from animals and humans with naturally occurring mutations in BMPs and related genes have shown that BMP signaling plays critical roles in heart, neural and cartilage development. BMPs are now considered to constitute a group of pivotal morphogenetic signals, orchestrating tissue architecture throughout the body (Bleuming *et al*., 2007).

TEST CHEMICAL: LDN193189

For the experimental study, the BMP receptors were targeted to inhibit the BMP signaling in the selected animal model the *P. latipinna*. The simple assumption behind it was if a chemical impedes or modulates an essential molecular target, then regeneration will be obstructed. Though there are many methods to block the BMP signaling, inhibition of pathway via synthetic inhibitor was preferred. One of the novel indolinone derivatives named LDN193189 (Figure 3) was selected for the study since it is a more potent and selective inhibitor of BMP signaling than other available inhibitors like dorsomorphin (Kamiya *et al*., 2010). Additionally, LDN193189 is a more selective reagent to assess the role of BMP signaling without interfering with VEGF or TGF- β signaling. Therefore, use of LDN193189 in the study was preferred over other chemical inhibitors of BMPR1 receptor.

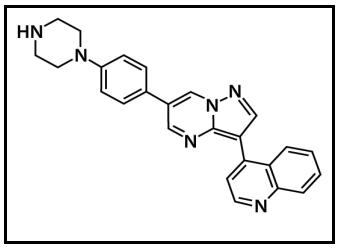

Figure 3. LDN193189, DM-3189, 6-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-3-(pyridin-4yl) pyrazolo [1,5-a] pyrimidine hydrochloride

MODEL ORGANISM: THE SAILFIN MOLLY (*Poecilia latipinna* Lesueur, 1821**)**

Teleosts are the largest group of ray-finned (actinopterygian) fish and include the majority of living fish species. Fin regeneration has been documented in a variety of species, including goldfish, trout, minnow and tilapia (reviewed by Akimenko and Smith 2007). Fish fin regeneration is reported to be dependent on BMP signaling (Crotwell *et al*., 2004). Therefore, it was thought relevant to examine the role of BMP signaling in the regulation of main milestones of epimorphosis in a teleost fish - sailfin molly, *Poecilia latipinna* (Lesueur, 1821) (Figure 4). It belongs to family Poeciliidae, comprising over 190 species (Parenti and Rauchenberger, 1989). The natural distribution of the sailfin molly is fresh, brackish, and salt waters of Florida, Mexico, Texas, South and North Carolina, and Virginia (Petrovicky, 1988; Courtenay and Meffe, 1989). Sailfin molly is a popular ornamental fish traded globally by the aquarium industry, and much of the demand for *P. latipinna* throughout the world is satisfied by its captive culture in India and elsewhere (Ramachandran, 2002; Ghosh *et al*., 2003). The sailfin molly is a fusiform shaped small fish (15-53mm total length) with a small head and upturned mouth (Ghosh *et al*., 2003). The dorsal fin is greatly enlarged in mature males compared to those of mature females. The sailfin molly is primarily an herbivore feeds on plants and algae, but occasionally it feeds on detritus or insect larvae (Meffe and Snelson, 1989). It has been used for research and for biological control of mosquitoes (Courtenay and Meffe, 1989). Previous work done on sailfin molly in our lab has shown the crucial role played by fibroblast growth factor-2 in epimorphic regeneration (Annasamudram, 2012)

Figure 4. Adult *Poecilia latipinna*

The selected test system is a simple, responsive and inexpensive vertebrate model to study molecular mechanism of caudal fin regeneration. Its ready availability with local animal suppliers, relative ease of maintenance and rapid growth post amputation were also deciding factors for its selection as animal model for the present study.

OBJECTIVE

It is apparent from the above review that BMP regulates a myriad of biological activities during embryonic and post-embryonic development**.** Hence, the overall objective of the present study was to elucidate whether BMP signaling induces a comparable response during fish fin regeneration as that of other vertebrate models and if yes then the potential role(s) the BMP signaling play during caudal fin regeneration in teleost fish *Poecilia latipinna*. To achieve this objective, a four-pronged parallel approach was proposed as described below using teleost fish with amputated tail fin, wherein BMP signaling was selectively inhibited with LDN193189 injection.

1. Study the role of BMP signaling in the initiation and progression of epimorphic regeneration.
2. Study the role of BMP signaling in the differentiation of skeletal and support tissues in the regenerate.
3. Study the role of BMP signaling in cellular synthetic and proliferative activities during regeneration.
4. Study the role of BMP signaling in apoptosis during tail regrowth.