INTRODUCTION

Regeneration is a fundamental attribute of all living things, whether it is simple tissue restoration or the complete replacement of lost body parts, such as limbs, tails or even heads As a biological problem, it began to be formally studied over 250 years ago in crustaceans by Rene-Antione Ferchault de Reaumur (1683 -1757), and soon after in *Hydra* by Abraham Trembly (1710-1784). In the years to come, regeneration attracted the attention of prominent biologists such as Charles Darwin (1809-1882) and Thomas Hunt Morgan (1866 –1945). A long-standing problem of biology, regeneration in metazoans still awaits a satisfactory mechanistic explanation The Italian Scientist Lazzaro Spallanzani (1729-1799) investigated in great detail the ability of various animals to regenerate missing body parts In his "*Prodromo di un opera da imprimersi sopra la riproduzioni. animali*" published in 1768, Spallanzani reported the regenerative properties of earthworms and snails, and of the legs, tails and jaws of aquatic salamanders, as well as tails of tadpoles and legs of the young and adult frogs Further, throughout the history of experimental biology, certain organisms have repeatedly attracted the attention of each new generation of researchers These "fascinating" animals are unique because they point out the potential of biological processes as they apply to humans. Urodele amphibians are members of this group because they alone, among the vertebrates, are able to regenerate lost body parts as adults They are a constant reminder that regeneration is an ancient and fundamental biological process, and they challenge our creative and scientific abilities to discover how to unlock the regeneration potential within us Though, the ability to regenerate is tremendous in invertebrates, among the vertebrates, the most extensive power of regeneration is shown by amphibians, wherein Urodeles can regenerate their limbs, tails, jaws and snout while Anurans can regenerate their hind limbs and tails in larval life. Among reptiles, only the tail of lizards and shell of testudines show extensive regenerative abilities Mammals, which are the highest form of life, lack any such capability of regenerating the organs once lost, but can regenerate some part of tissues like liver, muscle, bone, blood and epithelia (Uchida *et al.,* 2000)

Based on cellular mechanisms, regeneration can be divided into two broad categories: 1) morphallaxis and 2) epimorphosis Morphallaxis refers to the type of regeneration in which lost body parts are replaced by the remodeling of the remaining tissue Nearly all organisms that regenerate do so through the utilization of stem cells or progenitor cells In many cases

these stem cells are already present in the organism and need only be activated when the need arises. For example, *Hydra* utilizes stem cells found in the gastric region to regenerate itself or its lost structures (Bosch, 1998) and thus is considered immortal (Chatterjee *et al.,* 2001) This process is not dependent upon cellular proliferation In contrast to morphallaxis, epimorphosis requires active cellular proliferation prior to the replacement of the lost body part For example, planarians use pre-existing stem cells, known as neoblasts, to regenerate lost structures (Morgan, 1898; Morgan, 1901). This remarkable regeneration ability depends on the activation, proliferation and differentiation of neoblasts (New mark *et al.,* 2000) However, in vertebrates, epimorphic regeneration is the most prevalent form of regeneration and also the most widely studied In vertebrates with more extensive power of regeneration, such as salamanders, new stem cells or progenitor cells are created through a process of cellular dedifferentiation in which differentiated cells can reverse the normal developmental processes and once again become precursor cells (Thornton, 1938, Thornton, 1938; Chalkley, 1954, Bodemer *et al.,* 1959; Hay *et al.,* 1961; Lo *et al.,* 1993, Kumar *et al.,* 2000) In mammals, however, stem cells are activated during regeneration of muscle, bone, epithelia and blood and recently it has been demonstrated that certain regions of the adult mammalian central nervous system also contain stem cells (Uchida *et al.,* 2000).

The process of epimorphic regeneration has been widely studied in amphibians, where it occurs in several stages like. 1) preblastemal or wound epithelial stage; 2) blastemal stage; and 3) differentiation or morphogenetic phase Following limb amputation in amphibians, epithelial cells begin to migrate across the amputation site to form wound epithelium (WE), which is a few layers thick This WE thickens in response to continued epithelial cell migration and within days forms the mature Apical Epithelial Cap (AEC) (Christensen and Tassava, 2000). The internal stump cells underlying the WE/AEC begin to dedifferentiate in response to undefined signals found in the early limb regenerate (Chalkley, 1954; Thornton, 1957, Bodemer *et al.,* 1959, Hay *et al* , 1961, Thornton *et al.,* 1965; Steen, 1968; Lo *et al.,* 1993, Kumar *et al* , 2000). These dedifferentiated cells then proliferate to form a mass of progenitor and pleuripotent cells, known as regeneration blastema, which harbours the cells that will later redifferentiate to form the regenerated limb. Some of the dedifferentiated cells can even transdifferentiate and contribute to cell lineages other than the lineage of origin (Steen, 1968, Namenwirth, 1974) More recent experiments have clarified the degree of cellular plasticity that can occur during *Salamander* limb regeneration (Lo *et al.,* 1993, Kumar *et. al.,* 2000). Besides, several experiments have led to the conclusion that fully differentiated newt muscle cells can transdifferentiate into chondrocytes through a dedifferentiation/redifferentiation process and is consistent with the hypothesis that some dedifferentiated cells might be multipotent (Brockes and Kumar, 2002) Thus, regeneration proceeds by local reversal of differentiation of adult tissues to provide the proliferating

mesenchymal or epithelial progenitor cells of the regenerate (Brockes, 1994, Okada, 1991; Wallace, 1981) During limb regeneration in amphibians, the blastemal progenitor cells arise by dedifferentiation of mesenchymal tissues at the amputaiton plane (Hay 1959, Steen 1968; Namenwirth, 1974; Lo *et al.,* 1993)

Dedifferentiation is also evident during the regeneration of other structures and organs. For example, Salamander jaw regeneration requires the dedifferentiation of the same types of cells that dedifferentiate during limb and tail regeneration (Ghosh *et al*, 1995; Ferretti *et al.,* 1997). During regeneration of newt retina or lens, the pigmented epithelial cells of the retina or dorsal iris, respectively, dedifferentiate to form proliferating non-pigmented precursor cells that dedifferentiate to form the lost structure (Ito *et al.,* 1999) Similarly, during the early stages of spinal cord regeneration in amphibians, the ependymal cells that line the central canal of the spinal cord transform from an epithelial to a proliferating mesenchymal cell type that will later redifferentiate not only into ependymal cells but also into interneurons that comprise the gray matter of the regenerated spinal cord (Butler *et al*, 1967, O'Hara *et al.,* 1992). Moreover, in response to cardiac injury, newt cardiomyocytes dedifferentiate to a proliferative state where they disassemble their myofibrils, reenter the cell cycle, and proceed through mitosis and cytokinesis (Oberpriller *et al.,* 1971, Oberpriller *et al.,* 1974, Bader *et al.,* 1978, Badar *et al*, 1979, Oberpriller *et al.,* 1995; Neff *et al.,* 1996).

However, cellular dedifferentiation, a phenomenon central to epimorphic regeneration in newts, is not normally observed in terminally differentiated mammalian myotubes Mouse myotubes are incapable of reentering the cell cycle, unless they have been genetically altered or treated with myoseverin, a microtubule binding purine (Endo *et al.,* 1989; Iujvidin *et al.,* 1990, Schneider *et al.,* 1994; Tiainem *et al.,* 1996; Novitch *et al.,* 1996; Endo *et al.,* 1998; Rosania *et al.,* 2000) Ectopic expression of msx-1, which encodes a homeobox-containing transcriptional repressor, can induce mouse myotubes to dedifferentiate to mononucleated cells that posses the properties of stem cells (Odelberg *et al.,* 2000). Moreover, mouse myotubes when treated with newt regeneration extract, are induced to dedifferentiate (Christopher *et al.,* 2001), while cultured newt myotubes can dedifferentiate when transplanted back into the blastema of a regenerating newt limb (Lo *et al.,* 1993, Kumar *et al.,* 2000) Thus, it is unlikely that each cell type requires a unique factor or set of factors to initiate its particular dedifferentiation cascade A more parsimonious explanation is that the same factor initiates the dedifferentiation of all cell types However, the molecular and cellular mechanisms that govern epimorphic regeneration still remain incompletely defined.

In the early stages of epimorphic regeneration in amphibians, cells migrate into the center of the blastema and make contacts, allowing cell-cell interactions to happen (Gardiner *et al.,* 1986). It is possible that after amputation at a particular proximal-distal (PD) location, cells arise with the appropriate level-specific identity and then generate more distal identities by successive local interactions (Gardiner *et al.,* 1995, French *et al.,* 1976, Bryant *et al.,* 1981, Bryant and Gardiner, 1992, Bryant, *et al.,* 1993) The early dedifferentiation stage is exposed to a variety of signals from the wound epidermis and from the general injury response with retinoic acid (RA) (Viviano *et al.,* 1995), Hedgehog protein (Riddle *et al.,* 1993), and FGF (Boilly *et al.,* 1991) being some possible candidates, although the evidence for axial variation in expression in each case is either incomplete or absent Retinoic acid (RA) is known to respecify the PD axis during limb regeneration (Niazi and Saxena, 1978) and under certain circumstances to respecify the dorsoventral and anteroposterior axes (Kim and Stocum, 1986), furthermore, it can switch the identity of a tail blastema in some anuran tadpoles so that it gives rise to limbs (Mohanty-Hejmadi, 1993) Similarly, although the precise relationship between Hox gene expression and positional identity is not understood either for limb development or regeneration, the expression of HoxA9 and A13 in the mesenchyme is an important indication of local specification as early as 1 to 2 days after amputation (Gardiner *et al.,* 1995)

Regeneration is a complex phenomenon that requires input from a variety of factors like hormones, neural peptides, cytokines, growth factors, etc Neural influence on epimorphic regeneration has been widely studied The proliferation of the *Salamander* limb regeneration blastema is dependent on the presence of nerves. Singer (1952) demonstrated that a minimum number of nerve fibers must be present for regeneration to take place. It is thought that the neurons release mitosis-stimulating factors that increase the proliferation of the blastema cells (Singer and Caston, 1972, Mescher and Tassava, 1975) There are several candidates for these neural-derived mitotic factors, and each may be important Glial growth factor (GGF), known to be produced by newt neural cells, is present in the blastema, and is lost upon denervation When this peptide is added to a denervated blastema, the mitotically arrested cells are able to divide again (Brockes and Kinter, 1986) A fibroblast growth factor may also be involved FGFs infused into denervated blastemas are able to restore mitosis (Mescher and Gospodarowicz, 1979). Another important neural agent necessary for cell cycling is transferrin, an iron-transport protein that is necessary for mitosis in all dividing cells (since ribonucleotide reductase, the rate-limiting enzyme of DNA synthesis, requires a ferric ion in its active site) When a hindlimb is severed, the sciatic nerve transports transferrin along the axon and releases large quantities of this protein into the blastema (Munaim and Mescher, 1986, Mescher, 1992) Neural extracts and transferrin are both able to stimulate cell division in denervated limbs, and chelation of ferric ions from a neural extract abolishes

its mitotic activity (Munaim and Mescher, 1986, Albert and Boilly, 1988) The relationship between nervous system and regeneration was established long back since 1940's, by a number of workers (Singer, 1946, 1947, 1952, Butler and Schotte, 1941, 1949, Schotte and Butler, 1944, Van Stone, 1955, 1957, 1964). Kamrin and Singer (1955), Simpson (1964, 1965), and Cox (1969) showed nervous participation in the lizard tail regeneration. During early phases of tail regeneration in *Mabuya Carinata*, Acetylcholinesterase and Butyrlcholinesterase (AChE and BuChE) are negligible indicating that nervous stimulation is not essential for the initiation and formation of the early regenerates. However, activity of both these enzymes is higher during later phases of regeneration, suggesting their possible role in histo-differentiation of various tissues (Radhakrishnan, 1972) Though, a coordinated activity of all the factors, in time and space, is essential for truthful regeneration of the lost appendage, the maintenance of each stage is also mandatory to allow the regenerate to enter the next stage of regeneration uninterrupted However, there are many by-products of the metabolic pathways that can interfere with the biological processes, the most deleterious of them being reactive oxygen species (ROS).

In normal metabolic processes, partially reduced and highly reactive metabolites are formed during electron transfer reactions, which include superoxide anion (O_2) and hydrogen peroxide (H_2O_2). However, oxygen free radicals are continuously produced during aerobic metabolism (Halliwell, 1984), cells are protected from the toxic effects of these ROS due to antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and small molecules such as glutathione During early events of epimorphic regeneration, there are series of metabolic processes that happen at a faster pace, hence these antioxidant enzymes and molecules are of great significance in protecting the cells from potential damage and maintaining the status quo By and large excess ROS are detrimental to wound healing (Weiss, 1989) causing cell membrane and DNA damage, hence their detoxification is an essential step for successful healing of the wound Further, recent studies have shown that ROS are not only harmful to the cells, but are also involved in the cell signaling events, when present at optimum levels (Finkel *et al.,* 1998, Pryor *et al.,* 1991). Furthermore, after healing of the wound during epimorphic regeneration, the cells of the AEC divide repeatedly to contribute to the formation of blastema.

The proliferation of blastema cells requires a number of growth factors from the wound epidermis and the nerves, which regenerate into the tip of the amputated limb (Nye et al, 2003). The wound epidermis is thickened into an apical epidermal cap (AEC), which is functionally equivalent to the AER of amniote embryonic limb buds and which is essential for the survival and proliferation of the subjacent mesenchymal cells Growth factors expressed by the AEC and shown to promote blastema cell proliferation are Fgf-1, -2, and -8, while

neural growth factors that have similar effects are Fgf-2 and Glial growth factor-2 (Ggf-2) (Brockes, 1984). The neurotransmitter substance P and the iron-binding protein transferrin, as discussed earlier, are also neural factors that have a positive effect on blastema cell proliferation (Stocum, 2003) In addition, the blastema cells themselves express Fgf-10, which has been shown to be essential for limb regeneration in Xenopus (Yokoyama *et al.,* 2001). Thus, growth factors are a group of substances that are identified as potent regulators of various cell functions during normal physiological as well as regenerative processes. Many growth factors are known to play role during epimorphic regeneration viz epidermal growth factor, fibroblast growth factors, transforming growth factors, nerve growth factor, platelet derived growth factor, vascular endothelial growth factor, etc (Pilo and Suresh,. 1994,Kurup, 1996) Among the various growth factors involved in epimorphic regeneration, the role of Fibroblast Growth Factors (FGFs) has been studied at length in amphibians FGFs consist of a family of about twenty three members (FGF-1 to FGF-23), each consisting of a conserved core region of about 155 amino acids (Tanaka *et al.,* 2004) FGFs are small peptide growth factors with multiple biological functions which play significant roles in patterning, growth, and differentiation (Szebenyi and Fallon, 1999)

Of the growth factors, fibroblast growth factors are potent regulators of a variety of cellular processes including proliferation, differentiation, migration, morphogenesis, tissue maintenance and wound healing and repair (Clarke *et al.,* 1993, Cuevas *et al.,* 1988, Burgess and Maciag, 1989, Rifkin and Moscatelli, 1989) In addition, the fibroblast growth factors are potent inducers of DNA synthesis in a multitude of cells (Imamura *et al.,* 1990, Kan *et al.,* 1993, Lathrop *et al.,* 1985; Olwin *et al ,* 1986; Vainikka *et al.,* 1994; Wang *et al.,* 1994; Wiedlocha *et al.,* 1994) Besides, the cells of all the three germ layers during embryogenesis are responsive to FGFs in rats (Burgess and Maciag, 1989, Hou *et al.,* 1991) and in development of chick nervous system and mesoderm (Khot and Ghaskadbi. 2001). FGFs also stimulate cells to migrate chemotactically (Clyman *et al.,* 1994, Landgren *et al.,* 1998; Sa *et al.,* 1994) This is of importance both in angiogenesis and in wound healing (Burgess *et al.,* 1989) Further, FGFs stimulate cells to secrete proteases such as plasminogen activator (Mignatti *et al.,* 1989, Miralles *et al.,* 1998, Rusnati *et al.,* 1996), collagenase (Aho *et al.,* 1997, Hurley *et al.,* 1995; kennedy *et al.,* 1997; Mignatti *et al.,* 1989; Newberry *et al.,* 1997) and gelatinase (Weston *et al.,* 1996) Together, these FGF stimulated cellular functions, viz. cell proliferation, migration, and protease secretion, provide the basis for matrix reorganization and angiogenesis which are important physiological functions of FGFs FGFs also stimulate certain cells to differentiate Differentiation has been studied extensively in PC12 pheochromocytoma cells (Kimura *et al.,* 1994; Lin *et al.,* 1998, Marshall *et al.,* 1995, Raffioni *et al.,* 1992, Spivak-Kroizman *et al ,* 1994, Togari *et al.,* 1985) and FGF induces neurite outgrowth in these cells Similar effects have also been studied in other cell

lines (Kuo *et al.,* 1997) and in primary neurons (Hall *et al.,* 1996, Johnson *et al.,* 1993, Williams *et al.,* 1994, Williams *et al.,* 1995) Besides, FGFs can stimulate endothelial cells to form tubular structure when grown in special collagen cells (Kanda *et al.,* 1997) Lens epithelial cells differentiate into lens fiber cells under the influence of FGF *in vitro* (Chamberlain *et al.,* 1989) as well as *in vivo* (Robinson *et al* , 1995). On one hand FGFs are reported to induce differentiation of preadipocyte fibroblasts into adipocytes (Johnson *et al.,* 1993) and also induce differentiation of keratinocytes (Werner *et al.,* 1993), whereas on the other hand there are reports indicating the differentiation inhibiting ability of FGF such as myogenic differentiation of MM14 skeletal muscle myoblasts (Olwin *et al.,* 1992, Rapraeger *et al.,* 1991). Moreover, FGFs can also protect cells from undergoing apoptosis (Chow *et al.,* 1995, Guillonneau *et al.,* 1997, Hughes *et al* , 1993)

In epimorphic regeneration, fibroblast growth factors are known to play significant roles as well. For example, FGF-1 is stored in the epidermal cap during limb regeneration in amphibians and stimulates the proliferation of the underlying mesenchyme (Boilly *et al.,* 1991) Similarly, FGF-8 is one of the key molecules implicated in the initiation, outgrowth and patterning of vertebrate limbs In the regenerating limb, FGF-8 expression is noted in the basal layers of AEC and the underlying thin layer of mesenchymal tissue during blastema formation stages (Han *et al.,* 2001, Christen and Slack, 1997) Moreover, the regenerating capacity depends on the mesenchymal tissue and FGF-10 is likely to be involved in this capacity (Yokoyama *et al.,* 2000). FGF-8 and FGF-10 mediate epidermal-mesenchymal interaction required for limb bud outgrowth and these FGFs play critical roles not only in limb development but also in limb regeneration (Yokoyama *et al.,* 2000) The AER provides signals to maintain mesenchymal cells in a proliferative state, in the progress zone (PZ) of the limb bud (Rubin *et al.,* 1972; Wolpert *et al.,* 1975) However, exogenous FGF-8 can induce ectopic limbs (Crossley *et al.,* 1996, Vogel *et al.,* 1996, Ohuchi *et al.,* 1994, Cohen *et al.,* 1995) and replace the AER to support continued limb development (Mahmood *et al.,* 1995; Crossley *et al.,* 1996; Vogel *et al.,* 1996) In addition, limb patterning along the anterior-posterior axis requires an interaction between the zone of polarising activity (ZPA) and AER. Sonic hedgehog (Shh), a proposed mediator of polarizing activity (Riddle *et al.,* 1993) can be induced and maintained by FGF-8 in the absence of AER (Crossley *et al.,* 1996; Vogel *et al.,* 1996) and thus can mediate the patterning activities

Of all the FGFs, Fibroblast Growth Factor-2 (classically known as basic Fibroblast Growth Factor) (Yamashita *et al* , 2000), is the most influential and very important factor for epimorphic regeneration. Fibroblast growth factor-2 (FGF-2) is a member of the FGF family that comprises twenty three members (Tanaka *et al.,* 2004) This FGF prototype has

pleiotropic effects in different cell and organ systems. FGF-2 is a potent angiogenic molecule *in vivo* and *in vitro* stimulates smooth muscle cell growth, wound healing, and tissue repair (Basilico *et al.,* 1992, Schwartz *et al.,* 1993) In addition, FGF-2 may stimulate hematopoiesis (Bikfalvi *et al.,* 1994; Allouch *et al.,* 1995) and may play an important role in the differentiation and/or function of the nervous system (Logan *et al*, 1991; Baird, 1994, Unsicker *et al.,* 1992), the eye (McAvoy *et al.,* 1991) and the skeleton (Riley *et al.,* 1993, Fallon *et al.,* 1994). FGF-2 is a 155-amino acid protein However, four forms of FGF-2, a shorter and three longer forms have been isolated (Gaspodarowicz *et al.,* 1986, Klagsbrun *et al.,* 1987, Moscatelli *et al.,* 1987, Sommer *et al.,* 19897; Story *et al.,* 1987, Presta *et al.,* 1988; Presta *et al.,* 1989, Brigstock *et al.,* 1990) The shorter form or Low Molecular Weight (LMW) form is derived from 155-amino acid FGF-2 by proteolytic degradation (Klagsbrun *et al.,* 1987) while the origin of higher molecular weight forms (HMW)) (196, 201 and 210 amino acids) has been attributed to CUG codons, which act as initiation codons for higher forms and is 5' to AUG codon (Florkiewicz *et al.,* 1989, Prats *et al.,* 1989) The HMW forms are derived from the alternative translation initiation (CUG) codons and contain the complete LMW sequence in addition to an NH_2-terminal extension of varying lengths (Moscatelli *et al.,* 1987, Sommer *et al.,* 1987, Florkiewicz and Sommer, 1989). The different forms of FGF-2 have been associated with different cell functions and cellular compartmentalization. LMW FGF-2 is released by the cells and stimulates cell migration, proliferation, and FGF receptor down regulation through binding to surface receptors, while HMW FGF-2 forms are primarily localized in the nucleus and modulate cell proliferation (Bugler *et al.,* 1991; Mignatti *et al.,* 1991, Florkiewicz *et al.,* 1991, Quarto *et al.,* 1991; Renko *et al.,* 1991, Bikfalvi *et al.,* 1995).

However, FGF-2 is known to be present in the extracellular matrix, bound to FGF binding proteins (Wu *et al.,* 1991) and it is thought to be released from the cells by plasminogen activator-mediated proteolysis which provides a mechanism for the dissociation of biologically active FGF-2 heparan sulfate complexes from the extracellular matrix (Dahl *et al.,* 2000, Saksela *et al.,* 1990) Because of its high affinity for heparin and haparan sulfate glycosaminoglycans, significant amounts of FGF-2 are associated with the extracellular matrix of *in vitro* cell cultures (Vlodavski *et al*, 1987, Bashkin *et al.,* 1989, Flaumenhaft *et al.,* 1989) *In vivo*, FGF-2 has been detected in the basal lamina of blood capillaries, primarily at sites of vessel branching, and in the endothelium of the capillaries of some tumors (Folkman *et al.,* 1988, DiMario *et al.,* 1989; Cordon *et al.,* 1990), suggesting that endothelial cell derived FGF-2 may mediate angiogenesis with an autocrine mode of action (Sato and Rifkin, 1988; Mignatti *et al.,* 1991, Bikfalvi *et al.,* 1995, Klein *et al.,* 1996). This hypothesis is supported by the observation that FGF-2 has an autocrine effect on several cell functions required for angiogenesis, including proliferation, migration, proteinase production, and

integrin expression (Sato and Rifkin, 1988; Mignatti *et al.,* 1991; Bikfalvi *et al.,* 1995, Klein *et al.,* 1997)

The biological activity of FGF-2 is mediated through a dual receptor system consisting of four high affinity tyrosine kinase receptors and low affinity heparan sulfate proteoglycans located at the cell surface (Moscatelli *et al.,* 1987; Flaumenhaft *et al.,* 1989) Four major receptor families have been identified FGFR1, FGFR2, FGFR3 and FGFR 4 (Basilico *et al.,* 1992; Jaye *et al.,* 1992). These receptors share common features including a cytoplasmic conserved tyrosine kinase domain, a transmembrane domain, and an extracellular ligand binding domain. However, spliced variants do exist that differ in the composition of the extracellular ligand binding domain, which can contain two or three immunoglobulin (I_g)-like loops (Hou *et al.,* 1991; McKeehan *et al.,* 1993). The FGFs differ in their abilities to signal through the different FGF receptor variants, which is an essential mechanism for regulating their activities (Ornitz *et al.,* 1996) The FGF receptor families are well conserved because FGFR1 to FGFR4 have been identified in species as primitive as *Drosophilla, C elegans* and Medaka fish (Emori *et al.,* 1994). The major extracellular non-signaling molecules involved in FGF activity are heparan sulfate proteoglycans (HSPGs) and several groups have reported that heparan sulfates are required for FGF signaling (Yayon *et al.,* 1991; Klagsbrun *et al.,* 1991; Chua *et al.,* 2004; Qiao *et al.,* 2003; Ornitz *et al.,* 1992, Ornitz *et al.,* 1992; Rapraeger *et al.,* 1991, Olwin *et al.,* 1992, Rapraeger *et al*, 1994). Nevertheless, FGFs are found to be bound to the FGF-binding proteins and need to be dissociated from them for their activity. Thus, the association of FGF-2 with FGF-binding proteins in the blood or the matrix may influence FGF-2 activity and/or bioavailability (Dennis *et al*, 1989; Hanneken *et al.,* 1994; Hanneken *et al.,* 1995) Endogenous FGF-2 may play a role in cell growth, migration, and differentiation Sato and Rifkin (1988) and Biro *et al.,* (1994) demonstrated that migration of bovine capillary endothelial cells is inhibited by FGF-2 antibodies, implying that endothelial cell migration is affected by extracellular FGF-2

Furthermore, FGF-2 is known to exert pleiotropic effects on various organ systems including embryonic development, tumerogenesis and angiogenesis (Basilico *et al.,* 1992; Powers *et al.,* 2000) FGF-2 plays a crucial role in mesoderm induction in Xenopus (Slack *et al.,* 1987, Kimelman *et al.,* 1987, Kimelman *et al.,* 1989; Slack *et al.,* 1992, Cornell *et al.,* 1994, Labonne *et al.,* 1994, Schulte *et al.,* 1995, Gillespie *et al.,* 1992, Gatoh *et al.,* 1995; Isaacs *et al.,* 1992, Isaacs *et al.,* 1994) FGF-2 induces endothelial cell proliferation, migration, and angiogenesis *in vitro* (Basilico *et al.,* 1992), and regulates the expression of several molecules thought to mediate critical steps during angiogenesis These include interstitial collagenase, urokinase type plasminogen activator (UPA), plasminogen activator inhibitor (PAI-1), UPA receptor and β1 intergrins (Montesano, 1992, Mignatti *et al.,* 1993, Klein *et al*,

1993). FGF-2 stimulates smooth muscle cell proliferation (Schwartz *et al.,* 1993) and is also known to play role in hematopoiesis (Bikfalvi *et al.,* 1994, Allouche *et al* , 1995) as well as it is a stimulator of megakaryocytopoiesis (Bikfalvi *et al.,* 1994, Han *et al.,* 1992, Bikfalvi *et al.,* 1992; Bruno *et al.,* 1993; Avraham *et al.,* 1994). In addition, FGF-2 is a potent mitogen for human stromal cells and delays their senescence (Oliver *et al.,* 1990) The candidate cells in bone marrow that produce FGF-2 are the fibroblasts, the stromal layer, or the cells of hematopoitic lineages Likewise, Brunner et al, (1992) reported that FGF-2 is expressed in platelets, megakaryocytes, and granulocytes. Furthermore, several functions of FGF-2 in the nervous system have been anticipated. While FGF-2 acts *in vitro* on both astroglial cells and neurons, the mature oligodendrocytes are induced to dedifferentiate and to proliferate by FGF-2 suggesting a mechanism for regeneration of the oligodendroglial lineage after demyelination (Grinspan *et al.,* 1993) Also, neural precursors isolated from adult rat brain are induced to proliferate and to differentiate by FGF-2 (Richards *et al.,* 1992) Besides, FGF-2 may participate in a cascade of neurotrophic events facilitating neuronal repair and survival (Gomez-Pinilla *et al.,* 1992) Hence, changes in FGF-2 levels and/or its receptors are associated with several pathologies of the nervous system including the neurodegenerative diseases, Alzheimer's (Stopa *et al.,* 1990; Cotman *et al.,* 1991, Mattson *et al.,* 1993; Imaizumi *et al.,* 1993), Huntington's (Tooyama *et al.,* 1992) and Parkinson's (Tooyama *et al.,* 1993)

Moreover, changes in FGF-2 or FGF receptors may be involved in the genesis of certain brain tumors like glioblastomas and astrocytomas (Zagzag *et al.,* 1990; Yamaguchi *et al.,* 1994) Fast growing prostatic tumours exhibit high FGF-2 expression and several spliced variants of FGFR (McKeehan *et al.,* 1993). The proliferation and differentiation of normal human melanocytes are dependent on FGF-2 (Halaban *et al.,* 1992) Further, FGF-2 modulates basal and LH/Human choriogonadotrophin (LH/Hcg)-Stimulated leydig cell function (Murono *et al.,* 1992) In addition, FGF-2 has a very crucial role during healing of the wound (Kunta *et al.,* 1992; Gibran *et al.,* 1994, Tsuboi *et al* , 1992; Phillips *et al.,* 1993; Albertson *et al.,* 1993; Legrand *et al.,* 1993; Stenberg *et al* , 1991, Marks *et al.,* 1991, Pierce *et al.,* 1992; Chen *et al.,* 1992; Slavin *et al* , 1992) It is localized in the eye, retina, lens photoreceptors, aqueous and vitreous ocular media, and in the corneal epithelium (Consigli *et al.,* 1993; Schulz *et al.,* 1993). Goureau et al (1993) showed that FGF-2 induces nitric oxide synthase activity in retinal pigmented epithelial cells Thus, FGF-2 may participate in photoreceptor transduction, in part, by the regulation of nitric oxide production. FGF-2 also accelerates the healing of retinas and corneas (Schuschereba *et al.,* 1994, Fiddes *et al.,* 1991, Mazue *et al.,* 1991, Rieck *et al.,* 1993, Rich *et al* , 1992, Hoppenreijs *et al.,* 1994) It is also involved in skeletal muscle growth and differentiation. But, FGF-2 is an inhibitor of skeletal muscle differentiation and is operated by activating signaling pathways independent

of PDGF-signalling pathways (Kundla *et al.,* 1995) However, the proliferation and differentiation of osteoblasts are stimulated by FGF-2 (Globus *et al.,* 1988) It possibly plays role in genesis of muscular disorders, as plasma levels of FGF-2 are elevated in many muscular dystrophy patients but are undetectable in control patients (D'Amore *et al*, 1994) It also stimulates proliferation, differentiation and migration of several intestinal epithelial cell lines (Dignas *et al.,* 1994; Bikfalvi *et al.,* 1997, Burgess, 1998; Jones *et al.,* 1999; Werner, 1998). Besides, FGF-2 induces prostaglandin synthesis in a variety of cell types, often in context of angiogenesis or tissue healing (Kage *et al.,* 1999, Kawaguchi *et al.,* 1995, Majima *et al.,* 2000) and stimulates PGE2 synthesis in intestinal epithelial cell line via p38α-dependent (p38 MAPK) increase in COX-2 mRNA stability (Teresa *et al*, 2003)

Apart from such diverse functions of FGF-2 in normal physiology, it is a key player in epimorphic regeneration as well. The process of epimorphic regeneration requires remodeling of the destroyed tissue that happens after the loss of appendage, immediately after amputation. Along with the repair process, reorganization of the matrix, angiogenesis of the destroyed blood vessels from the stump into the newly formed wound epithelium, dedifferentiation of the adult stump cells and the regeneration of the nerves also starts. Angiogenesis is tightly regulated by several extracellular signals with one of the most relevant agents displaying angiogenic properties, and the first to be identified, is FGF-2 (Bikfalvi *et al.,* 1997) that binds to tyrosin kinase receptors (RTKs) and activates intracellular signaling cascades partially overlapping with those initiated by other factors, such as VEGF (Cross and Claesson-Welsh 2001) Phospholipase Cγ (PLCγ), which binds to phosphotyrosine 766 (Mohammadi *et al.,* 1991), is a direct substrate of FGF-2 receptor in many cell types, and stimulates the metabolism of phosphoinositides, releasing inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG), however, the extent of its involvement in FGF signaling in endothelial cells has been questioned (Wu, *et al.,* 2000, Javerzat *et al*, 2002). On the other hand, in vascular endothelial cells, FGF-2 receptor activation leads to the recruitment of several adapter proteins (FRS2, Grb2) followed by the indirect activation of ras and mitogen activated protein kinase (MAPK) cascade (Javerzat et al, 2002) Further, MAPK triggers a series of downstream events, including the activation of phospholipase A2 (PLA2). In fibroblasts and endothelial cells, one of the major signaling pathways activated in response to FGF-2 is the release of arachidonic acid (AA) via the recruitment of phospholipase A2 enzymes (Piomelli, 1993). FGF-2 is a potent stimulator of proliferation and movement and triggers AA metabolism (Fateur *et al.,* 1991; Bikfalvi *et al.,* 1997) In endothelial cells, AA release and the lipoxygenase (LOX) pathway play a critical role in vascular cell proliferation induced by FGF-2 and other mitogens (Dethlefsen *et al.,* 1994). Cell culture studies show that cell proliferation is induced by FGF-2 mainly through MAPK-cPLA2 pathway but PLC and PLD pathways are also involved (Antoniotti *et al.,* 2003). Thus,

both Cyclooxygenase (COX) and Lipoxygenase (LOX) pathways contribute to the proliferative effect of FGF-2 (Antoniotti *et al.,* 2003) FGF-2 is also known to be involved in neuronal regeneration. FGF-2 and EGF stimulate mitogenesis of cultured neuroprogenitor cells, whereas neurotrophin-3 and brain derived neurotrophic factor enhance neuronal differentiation (Temple *et al.,* 1995, Ghosh *et al.,* 1995, Vicario-Abejon *et al.,* 1995) Further, FGF-2 is up-regulated within the hippocampus after brain injuries (Yoshimura *et al*, 2001). Normally, FGF-2 does not have a signal sequence for cell secretion through the golgi apparatus (Dahl *et al.,* 2000, Friesel *et al.,* 1995, Saksela *et al.,* 1990) and it is probably released extracellularly only after cell damage. According to this hypothesis, it is speculated that FGF-2 plays a negligible role in the normal state, but with increasing damage, more FGF-2 is released and stimulates neurogenesis (Yoshimura *et al.,* 2001). Activin A, a member of TGF-β super family, regulates the neuroprotective action of FGF-2 *in vivo* (Tretter *et al.,* 2000) Hence, it is speculated that brain injury not only up regulates synthesis of FGF-2 intracellularly, but promote cell secretion and dissociation from extracellular matrix (Yoshimura *et al*, 2001).

FGF-2 is also involved in regeneration of nerves during epimorphic regeneration FGF-2 expression is induced in the regenerating spinal cord in amphibians early after tail amputation, and is expressed in the undifferentiated cells lining the ependymal canal from which the new cord will form (Zhang *et al.,* 2000). Its expression gradually decreases with the onset of differentiation and in the regenerated cord, as in the normal one, it is not detected in the ependymal cells, but is expressed in a subset of neurons. This suggests that FGF-2 plays a role both in the early stages of regeneration, possibly in the proliferation of neural progenitors and in the maintenance of the undifferentiated state (Ferretti *et al.,* 2001) Since rapid growth is characteristic of early stages of tail regeneration, FGF-2 in addition to being up-regulated in the regenerating spinal cord, is also expressed in a subset of blastemal cells and chondroblasts, in the basal epidermal layer and even in differentiating muscles in amphibians (Ferretti *et al.,* 2001) These results indicate that FGF-2 plays an important role in tail regeneration and is likely to be involved both in proliferation and differentiation of tail tissues This is also supported by the finding that expression of the four FGFRs is differently regulated during tail regeneration (Zhang *et al.,* 2002). Analysis of FGFR mRNA in regenerating blastemas by RT-PCR shows no change in the level of expression of FGFR2 and 3, whereas FGFR1 and FGFR4 mRNA are up-regulated following tail amputation. Unlike other FGF receptors, FGFR4 expression is restricted only to the ependymal tube (Ferretti *et al.,* 2001)

While later stages of limb regeneration share mechanisms of growth control and patterning with limb development, the formation of a regeneration blastema is controlled by early

events that are unique to regeneration (Endo *et al.,* 2004) The limb blastema produces Zone of Polarizing Activity (ZPA) as the signaling center of the anterior-posterior patterning as in the developing limb bud, it thus supports the notion that the limb regeneration recapitulates the limb development (Imokawa and Yoshizato, 1997) Despite many similarities between limb development and regeneration, an intriguing difference is that in developing embryos, limbs can form normally in the absence of nerves, whereas regeneration ceases if the limb is denervated in the early stages of regeneration (Wallace, 1981) Later stages of regeneration, like limb development, are nerve-independent D/x-3 (gene responsible for patterning) expression peaks at the stage when regeneration switches from nerve dependency to nerve independency and D/x-3 expression is sensitive to innervations in the nerve-dependent stages, but not at later stages (Mullen *et al.,* 1996). FGF-2 which is capable of replacing the function of the permissive epidermis in limb development (Niswander *et al.,* 1993, Fallon *et al.,* 1994; Taylor *et al.,* 1994), can restore *D*/x-3 expression in blastemas denervated at the nerve-dependent stage and, remarkably, can also permit regeneration of these denervated limbs Thus, FGF-2 is present in both, AEC and in nerves and levels of FGF-2 decrease in response to denervation This suggests that there is a link between innervation and the formation of an AEC that is able to sustain distal outgrowth (Mullen *et al.,* 1996).

During tail regeneration in Urodele amphibians such as *Axolotls,* all the tissue types, including muscle, dermis, spinal cord, and cartilage, are regenerated (Echeverri *et al.,* 2001) It is not known how this diversity of cell types is reformed with such precision Studies on limb regeneration suggest that there is considerable dedifferentiation and cell plasticity during the formation of blastema. Detailed histological studies of the fate of muscle fibers at plane of amputation suggested that muscle fibers loose their myofibrillar structure, their nuclei enlarge, and then bud off as mononucleate cells into the blastema (Thornton, 1938; Hay, 1959) The first experimental evidence to support their unusual "budding off" mechanism of producing progenitor cells was provided by Lo et al (1993) The population of blastemal cells consists of mainly the dedifferentiated muscle fibers (Maden, 1976, Tanaka, 1997, Kumar 2000). Moreover, Gardiner and Bryant (1996) have shown that one of the first proteins to be formed after amputation is FGF-2, which is a major regulator of the events happening during wound healing process It is known to switch on and off different set of genes required during the early events of tail regeneration viz Dlx-3, Msx, Hox genes, shh, etc. After the formation of wound epithelium, it is the key molecule present in ZPA. It has been observed that one of the biggest problem areas in regeneration is the formation of blastema (Gardiner *et al.,* 1996) The organisms that can form this can follow into next set of events to regrow the lost limb/tail. Thus, the regrowing limb/tail must produce a protein called FGF-2, which in turn helps turn on and off different set of genes.

Comparison of the events and the expression of genes, during development and regeneration in amphibians, suggest that cellular dedifferentiation is the key event in epimorphic regeneration and there is need for the complete understanding of the mechanism Such an understanding might one day be used as a method for enhancing the regenerative capacity in mammals, which will prove to be a milestone in the medical history. However, studies to identify genes and the key factors playing role in regeneration are been largely performed in amphibians. Since reptiles are higher in hierarchy and closer to mammals and the fact that some of them can regenerate lost body parts, makes them more suitable model for study of regeneration and to disclose the secret of regenerating lost appendages. Survey of literature illustrates that only few regeneration studies have been performed in reptilian system (Woodland, 1920, Huges and New, 1959; Moffat and Bellairs, 1964) The process of caudal autotomy in saurians and the subsequent regeneration of lost tail have been noted as early in 17th Century The histology of the tail during process of regeneration in lizards has been studied in 1960's and 1970's by various investigations in different species of lizards (Ali, 1941; Huges and New, 1959; Simpson 1965; Bellairs and Bryant, 1985). Iten and Bryant, (1976) have stated that the process of tail regeneration in lizards is comparable to that seen in Urodele amphibians Further, tail regeneration in lizards has been investigated from a wide variety of histological, physiological and biochemical perspectives (Cox, 1969, Moffat and Bellairs, 1964, Huges and New, 1959)

In India, however, some basic regeneration studies have been performed on the gekkonid lizard, *Hemidactylus flaviviridis*, *Hemidactylus brookie*, *Calotes versicolor*, and *Mabuya carinata* Several studies on lizard tail regeneration have reported profound metabolic and biochemical changes in the tail during regeneration (Shah and Chakko, 1967, 1969, 1972, Magon, 1970; Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975, 1976, Shah *et al.,* 1971, Hiradhar, 1972; Radhakrishnan, 1972, Ramachandran, 1972, Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974, Ramachandran *et al.,* 1975; Kumar *et al.,* 1993; Kumar and Pilo, 1994, Pilo and Kumar, 1995; Kumar *et al.,* 1995) Moreover, it has also been observed that in relation to regeneration, structural and physiological changes occur in other parts of the body as well (Kinariwala, 1977). Few studies on the role of some growth factors in epimorphic regeneration have also been carried out. While epidermal growth factor enhances the growth of regenerate in *Hemidactylus flaviviridis* (Pilo and Suresh, 1994), TNF and NGF are known to retard the process of tail regeneration (Kurup, 1997) The gekkonid lizard, *Hemidactylus flaviviridis* is the most studied reptilian model in India. The stages during tail regeneration in lizards are comparable to those seen in Urodeles viz wound epithelial stage, blastemic stage and differentiation stage (Iten and Bryant, 1976) While looking into the events that occur during each of the stages in the process of regeneration, it is seen that

in amphibians dedifferentiation of stump cells takes place (Brockes and Kumar, 2002), however in reptiles it has not been established yet. Hence several questions arise: does any dedifferentiation really happen during reptilian tail regeneration or there are stored stem or precursor cells that contribute to blastema? Similarly there may be other differences in the epimorphic regeneration between the reptilian and amphibian system that needs to be studied.

In order to understand the similarity, if any, that exists in the process of regeneration between amphibian and reptilian regeneration, it was thought worth trying the role of the fibroblast growth factor-2 that is imperative for amphibian regeneration (Gardiner and Bryant, 1996; Mullen *et al.,* 1996), in initiating and possibly maintaining the process of epimorphic regeneration in reptiles as well In the light of diverse functions of FGF-2 in wound healing, cell proliferation, differentiation and angiogenesis during amphibian regeneration, the present study was aimed at understanding the role of FGF-2 in tail regeneration in *Hemidactylus flaviviridis*. The intention of the present study, therefore, was to unearth the role of FGF-2 in tail regeneration *in vivo* The experiments were designed to understand the stage specific role of FGF-2 during tail regeneration in *Hemidactylus flaviviridis* (Chapter I) This was carried out in two stages firstly by administering extraneous FGF-2 and looking into its influence on progress of tail regeneration; secondly by blocking endogenous FGF-2 through administration of antiFGF-2 and then following the events of the regenerating tail. Further, the role of FGF-2 in proliferative activities was studied by looking into the nucleic acid content of the blastema at key events of epimorphic regeneration (Chapter II) Histofluorescence localization of nucleic acids was also done for confirmation. As reactive oxygen species are known to influence the healing of wound and also involved in some cell signaling cascades, the study was further extended by looking into the antioxidants' status in the regenerate at different stages of tail regeneration (Chapter III). The study also included the influence of epidermal growth factor on tail regeneration in *Hemidactylus flaviviridis* (Chapter IV) Since it is known to play role in wound healing and proliferation of epithelial cells, its influence on growth rate of blastema was studied. The involvement of EGF in cell proliferation was studied by estimating nucleic acid levels in regenerate and also histoflourescence of the nucleic acid in the regenerate at different stages of tail regeneration

Thus, in the current study it was studied that how FGF-2 influences the regeneration of tail in the gekkonid lizard, *Hemidactylus flaviviridis* Despite several studies showing a huge influence of FGF-2 on epimorphic regeneration in amphibians, the exact mechanisms by which FGF-2 shows its influence are not being worked out yet. Moreover, it is known that FGF-2 carries out most of its functions through MAPK-cPLA2 pathway, PLC and PLD pathways are also involved as reported by Antoniotti *et al.,* (2003) Hence, FGF-2 might

adopt different signaling pathways for a variety of functions it performs. Considering its crucial role in amphibian regeneration, there is a need for further insight into the mechanisms by which FGF-2 participates in the regenerative process of the autotomised tail of *Hemidactylus flaviviridis*