Introduction
All cellular life recognizes and adequately communicates with the external environment. Thus an increasing repertoire of extracellular signaling molecules matching with equally sophisticated intracellular mediators was the central feature for the evolution of metazoan life (Brivanlou and Darnell, Jr., 2002). How these extra-cellular signaling molecules control various intra-cellular signal transduction cascades leading to differential “gene expression” has been the subject of interest for the molecular geneticists as well as the cellular biochemists for a long time.

The eukaryotic transcriptional apparatus consists of a host of proteins that include, RNA polymerases, general transcription factors, coactivators, corepressors and chromatin remodelers like histone acetylases, deacetylases, kinases and methylases (Naar et al., 1998; Malik and Roeder, 2000; Jones and Kadonaga, 2000; Jenuwein and Allis, 2001; Naar et al., 2001). The orchestrated assembly of these proteins leads to the initiation of transcription and the resultant mRNA transcripts. Even though approximately 200 to 300 proteins that constitute the transcription machinery are crucial for the survival of the cells and organisms, the major role of transcription regulation is vested on a larger number of proteins (approximately 2000 to 3000 in number) called gene specific transcription factors (Brivanlou and Darnell, Jr., 2002). These site specific transcription factors recruit coactivators, chromatin remodelers and the transcription machinery to initiate gene specific transcription. As development and cell specialization occurs, the selection among this 2000 plus transcription factors for the regulation of cell specific gene expression involves (i) a repertoire of cell surface receptors, (ii) cascade of signal transducers and (iii) numerous assembly of cis-regulatory enhancer sequences that activate or repress transcription of various genes in a synchronized manner. Combinatorial use of these regulatory components ensures that the complete set of regulators for each gene is unique and thus the right protein is produced at the right amount at the right time.

A. Regulation of Gene Expression by Extracellular Signals

Modulators of gene expression such as cytokines, growth factors, hormones etc., bind to their cognate receptors located on the cell surface (or diffuse through the membrane and bind to the intracellular receptors) and activate sequence of events leading to changes in gene expression. Numerous signaling
molecules such as mitogen activated protein kinases (MAPKinases), Protein kinase C (PKC), Protein kinase A (PKA), Phosphatidyl inositol kinase (PI3 Kinase), Janus kinases (JAK) etc., have been implicated in transmitting the information generated by receptor stimulation to the nucleus. Generally, such signals are transduced by sequential phosphorylation and dephosphorylation of various signal transducing molecules and also by shuttling of proteins from cytosol to the nucleus. Over the past decade significant amount of information has accumulated on the divergence (as well as convergence) and specificity of various signal transduction pathways. In the following section, a number of such pathways especially relevant to cardiac myocyte functions are discussed.

A.1. Transcription Factor Activation by MAP Kinases

Mitogen activated protein kinases (MAPKs) are a family of evolutionarily conserved enzymes that respond to a plethora of extra-cellular stimuli and regulate gene expression leading to movement, metabolism, cell death, proliferation and differentiation (Posas et al., 1998; Chang and Karin, 2001; Kyriakis and Avruch, 2001). They are activated by MAPK kinases (MAPKKs), which in turn are activated by MAPKK kinases (MAPKKKs). MAPKKKs are controlled by the activation of cell surface receptors, by mechanisms that are poorly understood. MAPKinases phosphorylate a range of protein substrates including diverse enzymes, transcription factors, cytoskeletal proteins and downstream signal mediators such as MAPK-activated protein kinases (MAPKAPKs) and other kinases. In mammals, at least four parallel MAPK cascades exist viz., Extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 kinase and extracellular signal regulated kinase-5 (ERK5) that respond differently to distinct extracellular stimuli (Chang and Karin, 2001; Kyriakis and Avruch, 2001; Johnson and Lapadat, 2002). Furthermore, each MAPK subtype exists in multiple forms, reflecting the increased complexity of these signaling pathways during evolution. MAPKinase pathways are further diversified by various upstream MAPKKs viz., MEK1/MEK2, MKK3/MKK6, MKK4/MKK7 and MEK5. Although there are some cross-talks and cell-type specificities, the JNK and p38 MAPK cascades are more strongly activated by stress stimuli and inflammatory cytokines, where as the ERK pathway is strongly activated by polypeptide growth
Fig 1.1 Schematic representation showing Organisation of mammalian MAPK cascades (Yang et al., 2003).
factors through receptor tyrosine kinases (Kyriakis and Avruch, 2001). The BMK1/ERK5 pathway is activated by stress and mitogenic stimuli (Kyriakis and Avruch, 2001).

MAPK cascades control transcription at several levels, most notably by phosphorylating transcription factors, co-regulators, and also by initiating chromatin modifications along the inducible genes. Many transcription factors contain phosphorylation-dependent transactivation domains (TADs) and in a few cases, phosphorylation-dependent transcriptional repression domain. The best characterized example of the phosphorylation mediated activation of a transcription factor is CREB. CREB is phosphorylated on Ser-133 by a number of protein kinases leading to interaction with coactivators CREB-binding protein (CBP) and p300 ((Deak et al., 1998; Mayr and Montminy, 2001; Wiggin et al., 2002). In addition to promoting transcriptional activity by the recruitment of coactivators, phosphorylation can also lead to transcription factor activation by relieving the repressors (Yang et al., 2003). In addition to targeting specific transcription factors that bind to characteristic DNA elements in the promoters, MAPKs can also target components of the general transcription initiation machinery. For example ERK phosphorylates TFII-I leading to enhanced transcription from the c-fos promoter (Kim and Cochran, 2000). Increasing evidence suggests that certain gene regulatory factors are also indirectly targeted by MAPK signaling pathways. For example, acetylation of nucleosomes in the c-fos promoter region increases in response to the JNK MAPK signaling pathway (Alberts et al., 1998). In addition to histone modifications, gross nucleosomal remodelling can also be regulated by MAPK pathways. Phosphorylation of Sko-1 by the yeast p38-like MAPK Hog, promotes recruitment of the SWI/SNF and SAGA chromatin-modifying complexes (Proft and Struhl, 2002).

**A.2. Modulation of Transcription Factor Activities**

Gene specific transcription factors are regulated by several mechanisms viz., differential tissue distribution, alternative splicing, signal specific up- and down-regulation of transcript/protein levels, modulation of DNA binding by post-translational modifications, nucleo-cytoplasmic shuttling, selection of heterodimeric partners etc (Hill and Treisman, 1995; Karin and Hunter, 1995; Treisman,
Among various signal transduction cascades studied so far, MAPK pathways are of prime importance as they control gene expression almost at all these levels.

**A.2.1. Intracellular Shuttling**

Shuttling between nucleus and cytoplasm plays a major role in regulating transcription factor activities. A number of important transcriptional regulatory pathways governed by extracellular protein-receptor interactions require proteolysis for the delivery of the transcription factor to the nucleus. Many such proteolytic steps are governed by cytoplasmic serine phosphorylation. The nuclear factor kB (NF-kappa B)/Rel family of transcription factors are activated by this mechanism. A cytoplasmic inhibitor of active NF-kB called IkappaB binds to a subunit of NF-kB and blocks its nuclear localization signal (Baeuerle and Baltimore, 1996; Karin, 1999; Perkins, 2000). Phosphorylation of two serine residues in IkappaB leads to its proteosomal degradation, following which NF-kB moves to the nucleus (Karin, 1999; Perkins, 2000). Given the diverse stimuli that activate NF-kB, it is believed that there are several pathways that converge at IkappaB to mediate its activation.

Some transcription factors are activated at the cell membrane by the activation of cognate receptors followed by their nuclear translocation. The Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway activates transcription by this mechanism and does not involve any second messengers. Various cytokines and growth factors bind to transmembrane receptors followed by their dimerization through reciprocal SH2 (SRC homology 2) domain resulting in the activation of STAT proteins (from its latent state) by tyrosine phosphorylation (Darnell, Jr., 1997; Stark et al., 1998). Translocation of activated STATs into the nucleus allows site specific DNA binding and gene activation. Diverse protein kinases including several mitogen activated protein kinases (MAPKs) also phosphorylate STATs on serine residues, allowing further integration of various signaling pathways (Decker and Kovarik, 2000).

SMADs proteins are also activated by a similar mechanism except that they are activated by serine rather than tyrosine phosphorylation. SMAD family of transcription factors transduces signals for the transforming growth factor-beta
superfamily (TGFβ) of ligands. TGFβ initiates signaling by binding to and bringing together type I and type II receptor serine/threonine kinases on the cell surface. Subsequently receptor II phosphorylates the kinase domain of receptor I, which then propagates the signal through phosphorylation of SMAD proteins. Once activated, SMAD is released from the receptor complex and translocates to the nucleus and binds cognate sequences to activate transcription. Cross talk between SMAD and other pathways have also been reported. For example, Ras mediated activation of ERK results in phosphorylation of SMADs 1, 2 and 3 thereby attenuating their agonist induced nuclear accumulation thus affecting SMAD dependent transcription (Kretzschmar et al., 1997; Kretzschmar et al., 1999).

MAPK signaling pathways are implicated in regulating the cellular localization of a number of transcription factors. For example, the ERK pathway targets the repressor of ETS domain transcription factor ERF, leading to its exclusion from the nucleus (Le Gallic et al., 1999). Another group of transcription factors whose activity is regulated by nucleo-cytoplasmic shuttling are the members of the nuclear factor of activated T cells (NFAT) family (Rao et al., 1997). The NFAT4 and NFATc1 isoforms are phosphorylated by JNK, leading to their exclusion from the nucleus while their dephosphorylation by calcineurin reverses the process (Chow et al., 1997; Chow and Davis, 2000).

A.2.2. Modulation of DNA Binding Activities

Binding of transcription factors to gene promoters are often regulated (both positively and negatively) by phosphorylation-dephosphorylation mechanisms. Phosphorylation may induce dissociation of transcription factors from an inhibitory molecule followed by its DNA binding (as in case of NF-κB-IκB, (Perkins, 2000). Phosphorylation of the N-terminal transactivation domain of c-Jun by JNK promotes the dephosphorylation of another site close to the DNA-binding domain and thereby enhancing its DNA binding activity (Papavassiliou et al., 1995). The POU-domain transcription factor TCFβ1 is phosphorylated by JNK, leading to enhanced DNA binding (Kasibhatla et al., 1999). In response to growth factor stimulation, the ribosomal transcription factor UBF is
Fig.1.2 MAPK signalling regulates multiple aspects of transcription (Yang et al., 2003)
phosphorylated by ERK within its DNA-binding domain, preventing its interaction with DNA (Stefanovsky et al., 2001).

**A.2.3. Modulation of Protein Level**

Another mechanism for regulating transcription factor activity is to control its abundance. This can be achieved either by enhancing its expression levels or by increasing its stability. MAPK pathways regulate many transcription factors by these mechanisms of which the AP-1 family members c-Jun and c-Fos are best studied (Whitmarsh and Davis, 1996). JNK, ERK5 and p38 (depending on the stimulus) have been implicated in increased transcription of c-jun; (Han et al., 1997; Kato et al., 1997; Marinissen et al., 1999; Marinissen et al., 2001). Like c-jun, c-fos promoter also contains a number of elements that are targets for MAPK signaling (Whitmarsh and Davis, 1996). Transcription factor abundance can also be modulated by different mechanisms. For e.g. Activated JNK phosphorylates c-JUN, JunB, ATF2 and p-53 and thereby protects them from degradation and therefore enhancing their transcriptional activity (Fuchs et al., 1997; Musti et al., 1997). FOXO1, a member of Forkhead family of transcription factors, is phosphorylated by Akt, followed by its translocation from the nucleus to cytoplasm leading to its degradation (Van Der Heide et al., 2004).

**A.2.4. Modulation by the Fluctuations of Second Messengers**

Certain transcription factors are modulated by intermittent fluctuations in second messengers. Transcription factors of the NFAT family are heavily phosphorylated in resting cells. Upon stimulation by certain agonists resulting in fluctuations in internal Ca^{2+} concentration, NFAT molecules are dephosphorylated by calcineurin, translocate to the nucleus, and activate transcription (Hogan et al., 2003).

**A.3. Signaling Specificity and Gene Expression**

Signal specificity is essentially determined by the interactions between successive molecules in a signaling pathway. Association of the signaling components into relatively stable complexes is likely to play an important role in maintaining signal specificity. Even though the same JAKs may be activated by different receptors, the spectrum of STATs activated by particular receptor
stimulation is presumably governed by specific physical interactions among STATs, JAKs, and receptors (Aaronson and Horvath, 2002). A major determinant of signal specificity is the substrate specificity of the kinases involved, about which relatively little is known. The additional docking sites other than the substrate phosphorylation sites are important for MAPK substrate recognition. The JNK/SAPK isoforms encoded by differently spliced mRNAs appear to have different substrate specificity for Jun (Kallunki et al., 1994). Activation of a transcription factor through phosphorylation at multiple sites by the same kinase might also in principle provide mechanism by which signal specificity is maintained (Hill and Treisman, 1995). Finally, the DNA binding specificities of transcription factors themselves provide an important mode by which the targets for a signal pathway can be varied. Many factors bind DNA as heterodimers (with related proteins) with different DNA binding specificity (Hess et al., 2004). Different STAT dimers also have subtly different sequence specificities.

A. 4. Interactions Among Transcription Factors and Enhanceosome Assembly

In higher eukaryotes, the timing and magnitude of transcription of a gene is determined by an array of cis-regulatory elements located in the promoter. Activity of many promoters thus requires simultaneous involvement of multiple transcription factors. Studies of the IFN-β and TCR-α enhancers have revealed the mechanistic details of how enhancer organization and cooperativity (amongst transcription factors) mediate the assembly of the nucleoprotein complex called ‘enhanceosome’ (Grosschedl, 1995; Maniatis et al., 1998; Carey, 1998; Merika and Thanos, 2001).

A. 5. Cell Type Specific Transcriptional Regulation

Response of different cell types to various signals depends on its developmental history which manifests itself in two ways. Firstly, whether the target elements for transcription factors are in an open chromatin conformation (accessible), or in a condensed chromatin conformation (inaccessible). Secondly, combinatorial interactions between ubiquitously expressed and cell type specific transcription factors may also lead to differential gene activity (Hill and Treisman, 1995).
A. 6. Effects of Signal Strength or Duration

Duration and magnitude of the signal can also affect transcriptional output from the same pathway. It has long been recognized that transient and sustained signaling from the Ras/ERK pathway can lead to the different biological outcomes like proliferation and differentiation (Marshall, 1995). Transient ERK signaling leads to the induction of c-fos transcription but not its phosphorylation and c-Fos is rapidly degraded. However, upon sustained ERK signaling, c-Fos is phosphorylated and stabilized (Murphy et al., 2002; Murphy et al., 2004).

A.7. Attenuation of the Transcriptional Response

Transcriptional response to growth factor stimulation is usually transient. However, relatively little is known about how it is attenuated. Negatively acting factors may be present prior to stimulus or may be synthesized as part of the response. Inducible negatively regulating factors act either directly to reduce transcription factor activity or indirectly by down regulating the signal itself. For example, following degradation of IkB, NF-kB is translocated to the nucleus. Subsequently IkB is re-synthesized and sequesters NF-kB in the cytoplasm and thus the signal is attenuated (Baeuerle and Baltimore, 1996; Karin, 1999; Perkins, 2000). As yet, little is known about the identity and / or regulation of enzymes that inactivate transcription factors. Nonetheless, many of them are presumably phosphatases which counteract the kinases.

B. Adrenergic Receptors in Cardiac Myocytes

In response to a host of normal and disease-related stimuli, the sympathetic nervous system is activated which is essential for the maintenance of homeostasis. The physiological responses of sympathetic activation are mediated through the catecholamines (Norepinephrine and Epinephrine) acting on the adrenergic receptors (ARs). In failing heart, chronic catecholamine stimulation of ARs contributes to cardiac remodeling (structural adaptation by myocyte apoptosis and hypertrophy). Based on their pharmacological properties and molecular structures, Adrenergic Receptors are divided into three subfamilies: α1 Adrenergic Receptors (α1ARs, which include the subtypes α1A, α1B and α1D), α2 Adrenergic Receptors (α2ARs, which include α2A, α2B and α2C; (Civantos and Aleixandre,
Introduction

2001) and β Adrenergic Receptors (which include the subtypes β1, β2 and β3; (Hoffman B and Lefkowitz R, 1996). The heart expresses all three subtypes of α1 and βARs (Brodde and Michel, 1999; Ponicke et al., 2001). In the heart, the β1-AR is the most predominant subtype, comprising 75–80% of total β-ARs. In contrast, there are only a small number of α-ARs, with a ratio of β- to α-ARs of about 10:1 in the human myocardium (Hoffman B and Lefkowitz R, 1996). Myocytes do not appear to express α2ARs in most species (Xiang and Kobilka, 2003). Besides controlling cardiac contractile rate and force, both α and β ARs play a critical role in regulating blood pressure, airway reactivity and metabolic functions.

B.1. Adrenergic Receptor Signaling in the Heart

Adrenergic receptors belong to the superfamily of G protein-coupled receptors (GPCRs), which contain a conserved structure of seven transmembrane helices linked by three alternating intracellular and extracellular loops (Bockaert and Pin, 1999). When bound to their ligands, GPCRs are stabilized in an active conformation and stimulate heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) through their intracellular domains. Activated G proteins then dissociate into Ga and Gβγ subunits and amplify and propagate signals inside the cells by modulating the activity of one or more effector molecules, including adenylyl cyclases, phospholipases and ion channels. Thus, receptors that couple to G stimulatory (Gs) or G inhibitory (Gi) proteins respectively modulate the activity of adenylyl cyclase (AC) to generate the second messenger cAMP and subsequently activate cAMP-dependent protein kinase (PKA). Gq-coupled receptors also activate phospholipase C that in turn generates diacylglycerol (DAG) and inositol 1, 4, 5-triphosphate (IP3) as second messengers which in turn activate protein kinase C (PKC). The nature of the intracellular response to catecholamine stimulation therefore depends not only on the type of activated receptor and its expression levels, but also on the G protein it couples to and the intracellular pathways they activate.

B. 1. 1. β Adrenergic Receptor Signaling

Stimulation of β-adrenergic receptor pathways, results in the phosphorylation of an array of proteins involved in metabolic regulation, growth
control, muscle contraction, cell survival and/or cell death. In response to catecholamine stimulation, cardiac function is primarily controlled through β-ARs. Both β1- and β2AR subtypes couple to Gs and activate AC, resulting in elevated cAMP levels and subsequent activation of PKA (Brodde et al., 1995). PKA activation is a critical step in the mediation of contractility through phosphorylation of L-type calcium channels and phospholamban (regulating calcium influx and reuptake) (Xiao et al., 1999; Rockman et al., 2002). In addition to Gs, β2ARs also couple to Gi (Pertussis-Toxin sensitive) pathway (Xiao et al., 1995). Despite the dominant role of the Gs/AC/cAMP pathway in βAR signaling (particularly that of β1ARs), different subtypes of βARs are also capable of coupling to other G proteins, thereby activating more than one intracellular signaling pathway. PI3K constitutes a key downstream event of acute β2-adrenoceptor–Gi signaling that confines and negates the concurrent β2-adrenoceptor-Gs-mediated cAMP signaling (Xiao et al., 2004).

As opposed to the relatively well-understood role of β1ARs and β2ARs in regulating cardiac function, the role of β3ARs in the heart remains unclear. The ability of adrenergic receptors to activate multiple pathways through different G proteins and second messengers often imparts diversity to adrenergic receptor responsiveness under different patho-physiological conditions. Other factors such as receptor distribution, type of species and age (Kuznetsov et al., 1995; Sabri et al., 2000) also determine the effect adrenoceptors on cardiac function.

B. 1. 2. α Adrenergic Receptor Signaling

αAR signaling in the heart is diverse and involves activation of multiple signaling pathways that regulate cardiac output as well as growth responses. All three subtypes of α1ARs i.e., α1A, α1B and α1D are expressed in the heart while the main subtype being α1B (Michel et al., 1994; Ponicke et al., 2001; Piascik and Perez, 2001). According to a recent study, α1B AR is involved in regulation of contractile function and cardiac growth, whereas α1D AR is involved in regulating arterial blood pressure (Chalothorn et al., 2003). Essentially, all three subtypes of α1ARs that are expressed in the heart couple their signal transduction machinery primarily through the PTX-insensitive Gq/11 family, thereby leading to intracellular calcium mobilization (through a Gq/ PLC/PKC pathway) (Garcia-
Sainz et al., 1999). However, several lines of evidence also indicate that in addition to Gq/11, α1ARs are also associated with PTX-sensitive Gai pathway (Steinberg et al., 1985; Perez et al., 1993; Gurdal et al., 1997). α1ARs also have the capacity to couple to other effector molecules such as phosholipase D (PLD) and a number of calcium/calmodulin sensitive kinases (Llahi and Fain, 1992; Stull et al., 1990). Additionally, these receptors couple to numerous intracellular calcium mobilization pathways via voltage-dependent and independent calcium channels (Minneman, 1988).

There is increasing evidence that subtype-specific signaling of ARs in cardiac myocytes involves the association of receptors and down-stream signaling molecules in membrane microdomains (such as caveolae or lipid rafts), where signaling molecules are held together by multidomain scaffolding proteins (Steinberg, 2004).

B.1.3. Regulation of Receptor Function by Desensitization and Internalization

Repeated or sustained exposure to agonists often results in rapid attenuation of receptor responsiveness by a process termed desensitization which involves receptor phosphorylation, internalization (into intracellular compartments) and downregulation (reduced synthesis and/or degradation of existing receptors) (Ferguson, 2001). Stimulation of α- and β-adrenergic receptors by catecholamines leads to their phosphorylation either by second messenger-regulated kinases (PKA, PKC) known as heterologous desensitisation or by G protein-coupled receptor kinases (GRKs) known as homologous desensitization (Hausdorff et al., 1989; Pitcher et al., 1998; Penn et al., 2000). An important consequence of GRK mediated receptor phosphorylation is subsequent endocytosis of receptors into intracellular compartments (Claing et al., 2002). While βAR endocytosis has been studied extensively, mechanisms of αAR internalization are less defined. Evidences suggest that following exposure to agonist α1A ARs also undergo rapid endocytosis (Mhaouty-Kodja et al., 1999; Diviani et al., 2003).

Heart failure is accompanied by impaired β-receptor function caused by decreased number of receptors and their functional uncoupling (Bristow et al., 1982).
Uncoupling of β-adrenoreceptor is mediated by β-adrenoreceptor kinase-1 (βARK-1), which phosphorylates the receptor and thereby rapidly decreases its sensitivity. One of the most prominent characteristics of the failing heart is a marked desensitization and down regulation of βARs (Bristow, 1998).

B.1.4. Dimerization of Receptors

A rapidly growing body of evidence suggests that GPCRs also exist as homo- and heterodimers (George et al., 2002). Heterodimerization between β1- and β2-ARs in the heart may be an additional critical regulatory component in the overall response of the heart to stimuli. Interestingly, in addition to their interactions between same family members, adrenergic receptors also form oligomers with the members of other GPCR families including angiotensin II type I receptors (Barki-Harrington et al., 2003). Therefore, further understanding of the overall consequence of adrenergic receptor function in cardiac hypertrophy and failure requires analysis of the unique signaling properties of each adrenergic receptor, as well as its interactions with other receptors in the heart (Barki-Harrington et al., 2004).

C. Cardiac Hypertrophy, Apoptosis and Integration of Signals

In response to diverse load conditions (pressure, volume, etc.), heart muscle cells typically undergo either hypertrophy or apoptosis, a process collectively referred to as left ventricular remodeling. Cardiomyocyte hypertrophy is characterized by an increase in myocyte cell size but not the number (as post-natal cardiac myocytes are terminally differentiated). It is initiated by various endocrine, paracrine, and autocrine factors that stimulate a wide array of membrane-bound receptors. Their activation results in the triggering of multiple signal transduction cascades. Many of the signaling pathways implicated in cell proliferation are also attributed to hypertrophic growth of myocytes. G-protein coupled receptors play an important role in normal cardiac function as well as in the development of cardiac hypertrophy (Rockman et al., 2002) and the most important myocardial GPCRs include adrenergic, endothelin, angiotensin and muscarinic receptors. Myocyte hypertrophy is often maladaptive and is concomitant with myocyte death. Since cardiac myocytes have a very limited capacity of self-renewal, biological processes leading to myocyte hypertrophy and
apoptosis remains the center of attention for cardiovascular biologists (Beltrami et al., 2001; Beltrami et al., 2003). Emerging evidence also suggests that pathways involved in myocyte enlargement are also associated with strong cell survival signals. The balance between cell survival and apoptotic pathways thus play a major role in the transition from hypertrophy to ventricular dilation, an end stage clinical condition (Olson, 2004).

An intricate circuitry of signaling modules has been implicated in hypertrophy of cardiomyocytes (Hunter and Chien, 1999; Frey and Olson, 2003). A growing number of intracellular signaling pathways including G proteins coupled to specific receptors, low-molecular-weight GTPases (Ras, RhoA, and Rac), mitogen-activated protein kinases, protein kinase C, calcineurin, gp130-signal transducer and activator of transcription, insulin-like growth factor I, fibroblast growth factor and transforming growth factor β receptors have been characterized as important transducers of the hypertrophic response. These signaling pathways culminate in the nucleus with the posttranslational activation of a set of transcription factors. When activated (in the adult myocardium), these factors re activates a "fetal" cardiac gene program. Increasing evidence suggest that the aberrant expression of fetal proteins involved in contractility, calcium handling, and myocardial energetics eventually leads to maladaptive changes in cardiac function (Palermo et al., 1995; Miyata et al., 2000).

Number of studies has established that stimulation of αARs induces a hypertrophic response that is characterized by activation of immediate early genes, up regulation of contractile protein genes (Simpson, 1983; Izumo et al., 1988) and reactivation of embryonic genes (Knowlton et al., 1993; Karns et al., 1995). Knockout of the α1BAR prevents the development of hypertrophy induced by a chronic infusion of Norepinephrine (Vecchione et al., 2002). Overexpression of a constitutively active form of α1BAR induces a marked hypertrophic phenotype including increases in heart/body weight ratio (Milano et al., 1994). Studies in mice with a double knockout of α2A/α2C ARs display higher plasma levels of catecholamine and develop cardiac hypertrophy and reduced cardiac contractility (Hein et al., 1999).
Overexpression of β1ARs in mice causes hypertrophy and interstitial fibrosis in young animals and proceeds to cardiac dysfunction as the animal ages (Engelhardt et al., 1999; Bisognano et al., 2000). Overexpression of human β2ARs (60–100 fold) in the myocardium enhances cardiac function and even higher levels (200–350 fold) results in cardiomyopathy (Milano et al., 1994; Liggett et al., 2000).

It is thus believed that rather than a single signal transduction cascade regulating cardiomyocyte hypertrophy, an integration of multiple signal components involving interdependent and cross-talking signal networks eventually leads to a unified response (Molkentin and Dorn II, 2001).

C. 1. Gq/G11, Gs and Gi Signaling in Cardiac Hypertrophy

Gq presents the convergence point for several receptors such as α-adrenergic, angiotensin and endothelin receptors that have all been implicated in mediating the hypertrophic response (Wettschureck et al., 2001; Rockman et al., 2002). Transgenic overexpression Gq (Sakata et al., 1998) results in cardiac hypertrophy and subsequently leads to cardiomyopathy with depressed contractile function. Furthermore, knocking out Gq and G11 genes results in myocardial hypoplasia and embryonic lethality indicating an important role of these mediators in controlling cardiac growth (Offermanns et al., 1998). Paradoxically, Gaq stimulation not only induces cardiac hypertrophy but also cardiomyocyte apoptosis (Sabri et al., 2002).

Transgenic animals overexpressing Gas also increases myocardial collagen content, fibrosis, cardiomyocyte hypertrophy and apoptosis (Iwase et al., 1996; Geng et al., 1999).

Both cardiac muscarinic and β2-adrenergic receptors are also coupled to Gi, which directly oppose Gs-dependent signaling and inhibit adenylyl cyclase activity. In failing hearts, Gai content is increased by ~30%, and basal adenylyl cyclase activity is depressed by ~70% (Molkentin and Dorn II). Gai2 knockout/β2AR overexpressing mice developed a more pronounced cardiac hypertrophy and earlier heart failure compared to the β2AR overexpressing animals, indicating an essential protective role for Gai2 (Foerster et al., 2003).
Fig.1.3 Schematic diagram illustrating mechanisms by which adrenergic receptors regulate apoptosis in cardiac myocytes (Singh et al., 2001)
C.2. Small GTP-Binding Proteins and Hypertrophy:

Small G proteins provide a critical link between cell membrane receptors and various signaling pathways. Five families of small G proteins have been described (Rho, Ras, ARFs, Rab, Ran), each consisting of several members (Clerk and Sugden, 2000). The first small G protein implicated in cardiac hypertrophy was Ras. When constitutively expressed, \textit{Ras} is sufficient to induce a significant increase in cardiac mass (Hunter et al., 1995) whereas dominant-negative \textit{Ras} mutants prevented phenylephrine mediated increases in cell size and protein synthesis (Thorburn, 1994; Abdellatif et al., 1998). Ras signaling is coupled to multiple downstream effectors, including Raf, PI3K, Ral-GDS/Rac, MAPkinase and Calcineurin pathways, all of which participate in the hypertrophic response.

A number of mediators of hypertrophic signaling are also activated by Rho-dependent signaling. RhoA signaling thus stimulates the activity of transcriptional regulators Serum Response Factor (SRF) and GATA4 implicated in hypertrophy (Frey and Olson, 2003).

Constitutive activation of \textit{Rac} also induces hypertrophy (Sussman et al., 2000), whereas dominant-negative \textit{Rac} mutant (N17rac1) prevents Phenylephrine induced increase in protein synthesis as well as cardiomyocyte size.

Most recently, the Rab family of small G proteins has also been implicated in the development of cardiac hypertrophy. Overexpression of \textit{Rab} in transgenic mice is sufficient to induce cardiomyocyte hypertrophy \textit{in vivo} (Wu et al., 2001).

C.3. MAPK Pathways

Mitogen-activated protein kinase (MAPK) pathways are extensively involved in hypertrophy (Chien, 1999). Overexpression of MAPK phosphatase 1 (MKP-1), which inhibits all three major branches of MAPK signaling, blocked both agonist-induced hypertrophy \textit{in vitro} and pressure overload-associated hypertrophy \textit{in vivo} (De Windt et al., 2001). In response to agonist stimulation or cell stretching, ERK1 and 2 become activated both in cultured cardiac myocytes and in isolated perfused hearts (Zou et al., 1996; Punn et al., 2000). These observations have implicated ERK-1 and -2 signaling as regulators of the hypertrophic response. Transgenic mice expressing constitutively activated
On the other hand, the cardiac-specific overexpression of constitutively activated MEK5-ERK5 leads to an eccentric cardiac hypertrophy that progress to dilated cardiomyopathy and sudden death (Nicolet al., 2001).

JNK, another MAPKinase is essential for cardiac hypertrophy and dysfunction. In cardiomyocytes, mechanical stretching or agonist stimulation (such as endothelin-1, phenylephrine and angiotensin II) results in rapid phosphorylation of JNK (Komuro et al., 1996; Choukroun et al., 1998; Yano et al., 1998). Furthermore, MKK7 is sufficient to induce all features of cardiomyocyte hypertrophy when overexpressed in cultured cardiomyocytes (Wang et al., 1998). Conversely, adenovirus-mediated expression of a dominant-negative MKK4 mutant attenuates the hypertrophic response to endothelin I in vitro as well as pressure overload-induced hypertrophy in vivo (Choukroun et al., 1998; Choukroun et al., 1999).

P38α and p38β are the two isoforms of p38 expressed in the human heart (Jiang et al., 1997; Frey and Olson, 2003). Like other MAPKinases, p38 activity is also induced in pressure overload and agonist (endothelin I and phenylephrine) induced hypertrophy (Clerk et al., 1998). Several transcription factors such as MEF2 and NFAT3 involved in hypertrophic gene expression are phosphorylated by p38 (Han et al., 1997).

C.4. PKC and Cardiac Hypertrophy

Various studies implicated different PKC isoforms in the development of cardiac hypertrophy. Treatment of primary myocytes with phorbol esters such as PMA, activate PKC and mimic hypertrophic effects of phenylephrine (Henrich and Simpson, 1988). Mice overexpressing PKCα (the most abundant isoform of PKC in heart) have hypocontractile hearts while PKCα deficient mice have hypercontractile hearts (Braz et al., 2004). Transgenic overexpression of PKCβ in hearts also elicits cardiac hypertrophy (Bowman et al., 1997).

C.5. Calcineurin and cardiac hypertrophy

During recent years intracellular phosphatase calcineurin in conjunction with the transcription factors NFAT-1 (Nuclear factor of activated T cells) have
drawn considerable attention as a mediator of hypertrophic response. Calcineurin is a serine-threonine phosphatase that is activated by calcium-calmodulin pathway. Elevation in cytoplasmic calcium concentrations leads to the association of calmodulin with calcineurin followed by its activation (Olson and Williams, 2000). Calcineurin dephosphorylates transcription factors of the NFAT family resulting in its translocation to the nucleus followed by the activation of NFAT response genes. Constitutive activation of calcineurin in transgenic mouse hearts is sufficient to induce massive cardiac enlargement and eventually heart failure (Molkentin et al., 1998). A similar result is also obtained by the overexpression of a constitutively active NFAT3 mutant (Passier et al., 2000). Elevated levels of Calcineurin A have also been found in failed human hearts (Lim and Molkentin, 1999) and overexpression of MCIP1, an endogenous inhibitor of calcineurin, reduces cardiac hypertrophy in mice overexpressing a constitutively active form of calcineurin (Rothermel et al., 2001). Mice deficient in calcineurin Aβ display a 12% reduction in basal heart size and are largely resistant to diverse hypertrophic stimuli such as pressure overload and infusion of AngII or isoproterenol (Bueno et al., 2002).

C. 6. PI3K/Akt/GSK-3 Dependent Signaling and Cardiac Hypertrophy:

Among the other signaling events mediating hypertrophic response, is the PI3K (phosphatidyl inositol 3-kinase)/Akt/GSK3β (glycogen synthase kinase 3β) cascade (Matsui et al., 2003). This pathway is regulated through several transmembrane receptors such as the IGF-I receptors and G protein-coupled receptors (Shioi et al., 2002). Transgenic overexpression of constitutively active PI3K (or Akt) in the heart induces concentric hypertrophy (Shioi et al., 2000; Condorelli et al., 2002; Matsui et al., 2002). On the other hand, deletion of PTEN, which counteracts PI3K activity (by dephosphorylating Phosphatidyl Inositol [3, 4, 5] - Triphosphate) results in compensated cardiac hypertrophy (Crackower et al., 2002). PI3K/Akt pathway also acts as an inhibitor of apoptosis, thereby promoting cell survival. Akt phosphorylates several pro-apoptotic molecules such as Bad, caspase 9 and Forkhead box transcription factors (FOXOs) thereby inhibiting their pro-apoptotic role (Clerk et al., 2003, Tran et al., 2003). Akt also
Fig. 1.4 Hypertrophy and apoptosis are signaling cues both involved in the etiology of heart failure, even though traditionally perceived as separate entities. (van Empel and De Windt, 2004)
activates other anti-apoptotic proteins such as NF-kB, CREB, and p53 (Brunet et al., 2001; Mayo and Donner, 2001).

D. Transcriptional Regulators of Myocyte Hypertrophy, Survival and Death

Hypertrophic growth involves control at multiple molecular levels: transcription initiation, transcript elongation, protein translation and post translational stabilisation. An intricate web of interconnected signaling modules has been implicated in hypertrophy of postnatal cardiomyocytes (Hunter and Chien, 1999; Molkentin and Dorn II, 2001). These signaling pathways culminate in the nucleus with the posttranslational activation of a set of transcription factors, all of which have earlier roles in heart development. When activated in the adult myocardium, these factors reawaken a “fetal” cardiac gene program. Numerous upstream initiating triggers for this pathway have been identified, but relatively little is known of the transcriptional targets that ultimately integrate such signals.

Among the various transcription factors implicated in cardiac hypertrophy MEF2, GATA, NFAT and AP-1 family of transcription factors appear to play a major role. Also various other transcription factors viz., ATF, SRF, CREB, NFkB and STATs have been implicated in cardiac hypertrophy. A number of genes induced during cardiac hypertrophy possess functional GATA sites in their promoter region and cardiac-specific overexpression of GATA-4 or -6 leads to cardiac hypertrophy (Pikkarainen et al., 2004). There is general agreement that cardiac hypertrophy is triggered by abnormalities in calcium homeostasis within the cardiomyocyte (Olson and Schneider, 2003).

NFAT family of transcription factors which directly handles the cellular Ca^{2+} fluctuations are important regulators of cardiac hypertrophy and malfunction. Stress signaling in heart stimulates the transcriptional activity of MEF2 by causing the nuclear export of class II histone deacetylases (HDACs), which associate with MEF2 and suppress its activity (McKinsey et al., 2000). In the rat, CREB mRNA levels have been reported to be reduced after chronic stimulation with the β-adrenergic agonist, isoproterenol (Muller et al., 1995). Transgenic mice that express a dominant negative mutant form of CREB under the control of the cardiac myocyte-specific α-MHC promoter developed severe and progressive dilated cardiomyopathy that closely resembled both the anatomical and
physiological features of the human disorder (Fentzke et al., 1998). Transgenic mice expressing ATF3 under the control of the alpha-myosin heavy chain promoter have atrial enlargement, and atrial and ventricular hypertrophy (Okamoto et al., 2001). Heart failure patients typically show elevated levels of circulating TNF-α in the blood and have significant myocardial NF-kB activation (Wong et al., 1998). Over expression of STAT3 has been reported to amplify the features of hypertrophy and were attenuated under the condition with inhibited STAT signaling (Kunisada et al., 1998). A large body of evidence also indicates that SRF-containing complexes are the end point targets in pathways associated with conversion of hypertrophic stimuli to cardiac cellular response. The transgenic mice with cardiac specific expression SRF developed cardiomyopathy and exhibited and other features of cardiac hypertrophy (Zhang et al., 2001). Recent evidences indicate that members of AP-1 family of transcription factors may have a major role to play during the transition from cardiac hypetrophy to failure (Nadruz, Jr. et al., 2004; Wellner et al., 2005; Ricci et al., 2005).

E. AP-1 Family of Transcription Factors: Integrators of Disparate Signals

Activating protein-1 (AP-1) was one of the first mammalian transcription factors to be identified and much of our current knowledge about transcription factors came from the studies on AP-1 (Angel and Karin, 1991). AP-1 activity is induced by a plethora of physiological stimuli and environmental insults and in turn it regulates a wide range of genes involved in cellular processes including cell proliferation, death, survival and differentiation. Furthermore, in spite of increasing knowledge regarding the physiological functions of AP-1, many of its target-genes mediating these functions are not known yet. The ability of the AP-1 proteins to control such divergent biological processes can be attributed to their structural and regulatory complexity. Rather than being a single protein, AP-1 complexes belong to a sub set of a larger family of dimeric proteins characterized by a repertoire of evolutionarily conserved basic DNA binding domain in conjunction with a leucine zipper region motif (bZIP, the prototype members are Jun, fos, CREB, CREM, ATF and Maf ) (Shaulian and Karin, 2002). AP-1 complexes are primarily the heterodimers of Jun (c-Jun, JunB, JunD) and Fos (c-Fos, FosB, Fra-1 and Fra2) proteins. These proteins form homo- and heterodimers
Introduction

with varying dimerization potentials among different members (of the family and sub-family). For example, while Jun proteins can dimerize either with itself or with various Fos, CREB and ATF proteins, Fos proteins do not form homodimers and dimerize only with Jun family members. Each of these proteins are differentially expressed and regulated, thereby generating a discrete assembly of AP-1 complexes in a specific cellular context (Wagner, 2001). AP-1 complexes recognize either TPA (12-O-tetradecanoylphorbol-13-acetate) response elements (TRE: 5'-TGAG/CTCA-3') or cAMP response elements (CRE: 5'-TGACGTCA-3'). The composition of the leucine zipper is also responsible for the specificity and the stability of homo- and heterodimers (Wagner, 2001; Eferl and Wagner, 2003). In contrast to the bZIP domains, the transcriptional activation domains of many of these members are less characterized. While Jun, Fos and FosB are strong transactivators, JunB, JunD, Fra-1 and Fra-2 exhibit only weak transactivation potential. AP-1 activity in a specific cellular context can be modulated at multiple levels viz., transcriptional activation of individual Fos, Jun family members, mRNA stability, posttranslational modifications, their selection of dimeric partners and their interactions with other bZIP transcription factors (and cofactors) (Eferl and Wagner, 2003).

E.1. Signalling Pathways that Regulate AP-1 Activity

AP-1 activity is induced by growth factors, cytokines, neurotransmitters, polypeptide hormones, cell–matrix interactions, bacterial and viral infections, and an ever expanding variety of physical and chemical stresses (Shaulian and Karin, 2002). These stimuli activate various MAPKinases (ERK, JNK, and p38) that enhance transcription of c-jun and c-fos genes (Whitmarsh and Davis, 1996; Han et al., 1997; Kato et al., 1997). Serum and growth factors activate extra cellular-signal-regulated kinase (ERK) which translocate to nucleus and activate the ternary complex factors (TCFs) that bind to c-fos promoter and activate its transcription (Hill et al., 1994). Furthermore, the ERKs also phosphorylate Fra-1 and Fra-2 and enhance their DNA binding activity (as a dimer with Jun) (Gruda et al., 1994). Induction of AP-1 under cellular stress is mostly mediated by the JNK and p38 MAPK cascades (Chang and Karin, 2001). Once activated, the JNKs translocate to the nucleus, phosphorylate c-Jun within its N-terminal
transactivation domain (Ser63 and Ser73) and thereby enhance its transactivation potential (Karin, 1995). JNKs also phosphorylate and thus potentiate the activity of ATF-2, which heterodimerizes with c-Jun and bind to divergent AP-1 sites including that in the c-jun promoter and enhance its transcription (Gupta et al., 1995). Constitutive expression of activated oncogenes, such as Ha-ras, also results in elevated AP-1 activity, primarily through the persistent activation of ERK and JNK (Hibi et al., 1993; Derijard et al., 1994). Phosphorylation of transcription factors like MEF2C and TCFs by p38 MAPKinases also activate AP-1 (Han et al., 1997). While transcriptional upregulation of c-jun and c-fos has extensively been investigated, that for other jun and fos family members is poorly understood.

E.2. AP-1, A Master Switch in Proliferation, Differentiation and Transformation

Prototype members of the AP-1 family i.e., c-Jun and c-Fos were initially identified as the cellular homologues of retroviral oncoproteins v-Jun and v-Fos. Subsequent findings that growth factors and tumour promoters induce AP-1 activity initially linked AP-1 to cell growth and transformation. Later, a number of cell-cycle regulatory genes such as cyclin D1, cyclin A, cyclin E, p53 and p21Cip1 were identified as the bona fide targets of AP-1 (Shaulian and Karin, 2001; Shaulian and Karin, 2002). Differential expression of individual Jun proteins thereafter revealed their unique roles in cell cycle regulation (Hess et al., 2004). Inhibition of c-fos and c-jun expression by antisense RNA further established their roles in cell proliferation (and cell cycle progression, Shaulian and Karin, 2001). The tissue-specific inactivation of c-jun in mice has demonstrated that it promotes proliferation of fibroblasts, hepatocytes and keratinocytes (Behrens et al., 2002; Li et al., 2003; Zenz et al., 2003). A similar correlation between reduced fibroblast proliferation and smaller cell size was made for junD−/− mice (Thepot et al., 2000). AP-1 proteins bind to the cyclin D1 promoter and activate its expression in transient transfection experiments. Global gene expression analyses have identified a number of growth factors, cytokines and chemoattractants as the targets of AP-1, thereby indicating that AP-1 induces cell proliferation through the induction of paracrine effectors (Florin et al., 2004).
Cells of lymphoid origin derived from transgenic mice over-expressing either JunB or JunD respond poorly to mitogenic stimuli thereby indicating that JunB and JunD can act as negative regulators of cell proliferation (Meixner et al., 2004; Bakiri et al., 2002). Overexpression of JunB antagonizes the c-Jun mediated induction of cyclin D1 in fibroblasts (Passegue and Wagner, 2000). Recent evidence indicates that the differences in JunB and c-Jun function may be cell-type-specific and also depend on their relative expression levels. In mice lacking JunB or ATF-2 expression, altered expression of cyclin A and impaired proliferation of chondrocytes and osteoblasts were reported (Beier et al., 2000; Hess et al., 2003; Kenner et al., 2004). Immortalized JunD −/− cells showing increased proliferation and higher cyclinD1 expression has been reported (Weitzman et al., 2000).

Fibroblasts deficient in either c-Fos or FosB proliferate normally, thereby indicating a redundant role. However, cells lacking both proteins have a reduced proliferative capacity (Brown et al., 1998; Brusselbach et al., 1995).

The importance of the dimerization partner of c-Jun for its transformation-potential has also been investigated. While c-Jun–ATF2 heterodimers induce autocrine growth, c-Jun–c-Fos heterodimers induce anchorage-independent growth (van Dam et al., 1998). However, the target-genes responsible for these activities remain to be identified.

The members of AP-1 family also participate in the differentiation and specialization of various cell types. JunB is required for efficient osteoclast differentiation as in animals lacking JunB results in decreased osteoclast formation (Kenner et al., 2004). JunB and JunD also control the differentiation and function of T helper (Th) cells mainly by regulating specific cytokines (Hartenstein et al., 2002; Meixner et al., 2004). A stem cell-specific role for JunB in normal and leukemic hematopoiesis has been reported (Passegue et al., 2004).

Recent studies have also elucidated a crucial role for Fos in bone morphogenesis. Exogenous expression of Fos in osteoblast cultures accelerates S-phase entry by deregulating cyclin A and cyclin E expressions and also by altered cyclin A/E CDK2 activity (Sunters et al., 2004). Overexpression of Fra-1, Fra-2 in transgenic mice leads to both increased bone formation and development of osteosclerosis or
lung and epithelial tumours (Eferl and Wagner, 2003). Mice lacking Fra-1 develop osteopenia (a low-bone-mass disease) and have long bones containing reduced levels of bone matrix components (Eferl et al, 2004). Fos is also involved in osteoclastogenesis as Fos deficient animals lack osteoclasts and develop osteopetrosis (Johnson et al., 1992; Wang et al., 1992). The osteopetrotic phenotype can be partially rescued by expression of Fra-1 (Fleischmann et al., 2000).

E.3. AP-1 as Regulators of Survival and Apoptosis

Paradoxically, AP-1 has been implicated in both induction and prevention of apoptosis and the exact outcome is highly tissue and developmental stage specific. Neuronal and lymphoid cells upon withdrawal of growth factors undergo apoptosis that is preceded by the induction of AP-1 (Estus et al., 1994). Cells upon exposure to genotoxic stress such as alkylating agents or short-wavelength UV radiation shows a robust induction of c-Jun followed by the onset of apoptosis (Devary et al., 1991; Derijard et al., 1994; Karin, 1998). It is suggested that the contribution of AP-1 to cell fate depends on the cellular context and the stimulus. Numerous studies have highlighted an important role for JNK in the control of lymphoid, fibroblast and neuronal cell death (Eichhorst et al., 2000; Kasibhatla et al., 1998; Derijard et al., 1994; Karin, 1998; Le Niculescu et al., 1999). Activated JNK phosphorylates Jun, which results in enhanced transcription of genes such as FasL and TNF-α implicated in apoptosis. However, under certain circumstances, JNK activation can also signal cell survival as it has been reported that JunD stimulated by JNK promotes cell survival after TNF treatment (Lamb et al., 2003). Inactivation of Jun by a dominant-negative mutant reduces expression of Bim, an apoptosis regulatory protein of the Bcl-2 family, and inhibits mitochondrial cytochrome c release (Whitfield et al., 2001). Motor neurons undergo atrophy due to reduced cell death in mice that do not express Jun in the nervous system (Raivich et al., 2004). On the other hand, spontaneous apoptosis occurs in absence of Jun in fibroblasts (from c-Jun–/– mouse), keratinocytes and notochordal cells (Shaulian et al., 2000; Behrens et al., 2003; Zenz et al., 2003). Overexpression of Fos in hepatocytes also inhibits cell-cycle progression and
Fig 5. Functions of AP-1 subunits in various cellular processes and disease. Antagonistic (indicated by the double-headed arrow) or common tasks for AP-1 members are observed in various cell types. Positive, negative or even dual functions of individual subunits influence pivotal cellular processes, such as differentiation, cell-cycle regulation or apoptosis. Deregulation of these processes by alterations in either single or multiple AP-1 subunits, thereby modulating AP-1 net activity, can be fatal for the cell and organism. (Reproduced from Hess et al., 2004).
stimulates cell death (Mikula et al., 2003). Fos is also a mediator of Myc-induced cell death in hepatoma cells deprived of growth factors (Kalra and Kumar, 2004).

AP-1 members are also involved in cell survival. For example, during Kainic-Acid-induced seizure, Fos expression is negatively regulated along with increased neuronal cell death (Zhang et al., 2002). During mouse development, Jun expression is required for the prevention of apoptosis in foetal hepatocytes (Eferl et al., 1999). Moreover, foetal liver apoptosis can be rescued by a JunB knock-in allele (Passegue et al., 2002). Thus the precise function of Jun in hepatocytes depends on their state of differentiation. While foetal hepatocytes require Jun for survival, differentiated hepatocytes require it for cell-cycle progression (Behrens et al., 2002; Eferl et al., 1999). Enhanced apoptosis (and proliferation defect) is also observed in keratinocytes that do not express Jun (Zenz et al., 2003). Recent studies demonstrate that JDP-2 (Jun dimerization partner-2), a newly identified member of the AP-1 family, protects fibroblasts against UV induced apoptosis by suppressing p53 at the level of transcription (Piu et al., 2001).

Thus the precise role of AP-1 in cell death or survival remains enigmatic. It appears that the relative amounts of different AP-1 proteins, the cell lineage, its state of differentiation, type of stimulus and the microenvironment contributes to how AP-1 modulates the decision of a cell to proliferate, differentiate or die (Hess et al., 2004). One interesting hypothesis in this regard is that AP-1 functions as a homeostatic regulator that keeps cells in a certain proliferative steady state and a tilt in balance triggers cell death (Shaulian and Karin, 2002). In spite of enormous studies on AP-1 proteins, only a handful of AP-1-target genes have yet been unambiguously identified. Thus, further identification of target genes, specifically those that are exclusively controlled by distinctive AP-1 complexes, will help us defining their precise role in cell death or survival.

E. 4. AP-1 in Cardiac Myocyte Hypertrophy and Apoptosis

Development of cardiac myocyte hypertrophy involves both quantitative and qualitative changes in gene expression such as recapitulation of an embryonic pattern of gene expression and an increase in contractile protein content that is initiated by the transient induction of immediate-early transcription factors like AP-1, Egr-1 and Myc (Komuro and Yazaki, 1993). A number of hypertrophy-
associated genes, for example, atrial natriuretic factor, skeletal α-actin and ventricular myosin light chain 2 contain AP-1 binding sites (TRE) in their promoters (Shubeita et al., 1992). Disparate hypertrophic agonists like Isoproterenol, Phenylephrine, Endothelin, Angiotensin, Norepinephrine, pressure/volume overload as well as end stage heart failure induces AP-1 in a consistent manner (Clerk et al., 2002; Frantz et al., 2003; Taimor et al., 2004). However the role played by AP-1 transcription factor(s) in cardiac muscle hypertrophy is largely unknown.

Enhanced expression and phosphorylation of Jun under acute pressure overload (leading to cardiac hypertrophy) has been documented (Nadruz, Jr. et al., 2004). Furthermore, one of the major downstream target of JNK is Jun protein and amongst its two isoforms, JNK1 has been implicated in various models of cardiac hypertrophy (Baines and Molkentin, 2005). Induction of c-Jun by various extracellular agonists like Endothelin, Phenylephrine, Angiotensin has also been reported in culture models of cardiac hypertrophy (Clerk et al., 2002). Transfection of a dominant negative mutant of c-Jun has been shown to inhibit cardiac myocyte hypertrophy induced by phenylephrine and endothelin further suggesting that c-Jun might play an important role in inducing cardiac hypertrophy (Omura et al., 2002).

As a transactivator, JunB is less active than Jun and it antagonizes Jun functions (Passegue et al., 2002). JunB is thus less effective (than Jun) in inducing the hANP promoter, a marker of hypertrophy (Kovacic-Milivojevic et al., 1996). Also anti-hypertrophic agonist ATP induces JunB expression in primary myocytes (Zheng et al., 1994). Although the role of JunD, the third member of the Jun family in cardiac myocyte hypertrophy has not been investigated, decreased expression of JunD in failing myocardium has been reported (Pollack et al., 1997). Mice lacking JunD demonstrate decreased expression of hypertrophic markers (ANP, BNP) after mechanical pressure overload. On the other hand, cardiomyocyte-specific expression of junD in mice results in ventricular dilation and decreased contractility (Ricci et al., 2005).

Role of c-Fos in cardiac hypertrophy has also been documented. Various neuro-hormonal factors like Angiotensin, Endothelin, Isoproterenol; mechanical factors
like pressure overload and stretch induces c-fos mRNA in cardiac myocytes (Barka et al., 1987; Komuro et al., 1990; Izumo et al., 1988; Neyses et al., 1993). Induction c-fos mRNA was reported in isolated perfused rat hearts after norepinephrine infusion (Kolbeck-Ruhmkorff and Zimmer, 1995) and also in experimentally induced myocardial infarction (Gidh-Jain et al., 1998).

There are conflicting results regarding the role of Fra-1 if any, in cardiac hypertrophy. While one study demonstrated that fra-1 is not induced upon aortic banding (Ricci et al., 2005) another study reported both repression and induction of fra-1 (in cardiomyocytes in vitro) based on the stimuli (van Wamel et al., 2000). Nonetheless transient transfection assay have demonstrated that fra-1 can down regulate ANF promoter activity (a marker of hypertrophy) (Kovacic-Milivojevic and Gardner, 1995). Furthermore, expression microarray analysis shows transcriptional up regulation of fra-1 during experimentally induced terminal heart failure models (Wellner et al., 2005). According to a recent study, fra-1 conditional knock-out mouse have a normal hypertrophic response, while hearts from fra-1 transgenic mice decompensate prematurely during pressure overload (Ricci et al., 2005). Moreover, transgenic overexpression of Fra-1 in mice lacking junD (JunD-/-) develops dilated cardiomyopathy with increased cardiomyocyte apoptosis (Ricci et al., 2005). Role of Fra-2 in cardiac hypertrophy, if any is not known.

Paradoxically number of pathophysiological stimuli leading to cardiac myocyte apoptosis also induces AP-1 as an immediate early response and at least some of those studies have also assigned pro-apoptotic role(s) for JNK and p38 MAPkinases (Baines and Molkentin, 2005). It thus appears that c-Jun protein (a substrate for JNK) may have a major role in inducing cardiac myocyte apoptosis while the roles of other Jun/Fos family members are largely unknown.