Cardiac muscle structure and function

The structure of cardiac muscle

The capacity for cells to utilize biochemical energy to generate both mechanical force and movement of the human body is a dominant feature found in muscle cells. There exist three distinct categories of muscle tissue, each differing by specific structural and functional properties. These categories include smooth muscles, skeletal muscles and cardiac muscles. Smooth muscles are involuntarily contracting, non-striated muscles that surround the inside walls of hollow organs such as the urinary bladder, reproductive organs, and both the gastrointestinal and respiratory tracts. Its contraction enables and regulates the progression of liquid content, such as food, urine and blood, along the internal passageways. Skeletal muscles are voluntarily contracting, striated muscles that attach to bones of the skeleton. The contraction of skeletal muscle is primarily responsible for the movement of the skeleton, but also has roles in heat production and protection of internal organs. Cardiac muscles are an involuntarily contracting, striated muscle found exclusively in the walls of the heart, more specifically in the myocardium. Contraction of cardiac muscles propel oxygenated blood into the circulatory system to deliver oxygen to the body, as well as regulates blood pressure (Martini et al., 2009).

Cardiac muscle tissue is composed of a network of individual cardiac muscle cells, called cardiomyocytes. Cardiomyocytes are small in size, averaging 10-20µm in diameter and 50-100µm in length, have a single centrally positioned nucleus and connect to adjacent cells in a branched manner through specialized sites known as intercalated discs (Martini et al., 2009). Two structures that are found within the intercalated discs are desmosomes and

gap junctions. Desmosomes are specialized structures involved in cell-to-cell adhesion and gap junctions are intercellular channels that connect the cytoplasm of adjacent cells, allowing the free passage of molecules, ions and electrical signals.

Within the cytoplasm of striated muscle cells are long, cylindrical organelles termed myofibrils. With a diameter of 1 to 2µm and numbering between hundreds to thousands in a cell, myofibrils are enveloped and grouped together by connective tissue called the fasciculus, which forms bundles of myofibrils that spans the length of the cell (Widmaier et al., 2006). Individual myofibrils can be further divided into two types of contractile filaments: thin filaments and thick filaments. These filaments are composed primarily of actin and myosin proteins, respectfully. The thin and thick filaments are aligned in a manner where they form repeating structural units along the length of the myofibril. Among these structures is the sarcomere, which is a Ca2+-dependent contractile unit responsible for muscle contraction and relaxation (Widmaier et al., 2006). An increase in cytoplasmic Ca2+ influx causes the thin and thick filaments to overlap each other, causing a shortening of the sarcomere, leading to a muscle contraction. Alternatively, a decrease in cytoplasmic Ca2+ levels causes the thin and thick filaments to pull away from each other, leading to relaxation of the myofilaments. The specific arrangement of the thin and thick myofilaments is responsible for the striated appearance of both skeletal and cardiac muscle tissue.

Electrical stimuli, called action potentials, are required for striated muscle cell contraction. In skeletal muscles, action potentials are derived from neurons in the brain and spinal cord that transmits the signal through the

nervous system and innervates muscle fibers, causing contraction. However, unlike skeletal muscles, the contraction of cardiac muscles occurs without neural stimulation, a property called automaticity (Martini et al., 2009). This is because the heart contains pacemaker cells, which are specialized cells that have no contractile function; rather having the ability to initiate and conduct action potentials to neighboring cardiomyocytes. The cardiac action potential propagates across cardiomyocytes through gap junctions, allowing the cells to contract in tandem, which enables the heart to contract as one muscle. Cells which have pacemaker activity constitute 1% of cardiac muscle cells, whereas the other 99% are contractile cells (Sherwood, 2006).

The conversion of an electrical stimulus into a mechanical response is performed through a physiological process known as the excitation-contracting coupling or the ECC. This phenomenon has a critical role in muscle cells as it allows a propagating action potential to cause shortening of the sarcomere, leading to muscle cell contraction. When action potentials are produced by pacemaker cells, they conduct across the heart by traveling along the length of the myofibril on the muscle sarcolemma. An action potential will transmit on the sarcolemma until it reaches a transverse-tubule (T-tubule). T-tubules are defined as deep invaginations into the sarcolemma that contact the cisternae of the sarcoplasmic reticulum (SR), an organelle that functions as a Ca2+ storing body. Upon penetrating the T-tubules, the action potential will cause a depolarization of the membrane voltage potential, leading to an increased influx of Ca2+ into the cytoplasm. Resting within the T-tubules are many ion transporters such voltage-gated L-type Ca2+ channels and Na+ / Ca2+ exchangers (D. Bers, 2002). These Ca2+-

transporters are opened/activated when stimulated by action potentials, prompting the entry of extracellular Ca2+ into specific microdomains in the cytosol (Berridge, 2006). An elevation of cytoplasmic Ca2+ levels will trigger the opening of ryanodine receptors (RyR), which are intracellular Ca2+ channels present on the membrane of the SR, allowing stored Ca2+ to exit the SR and enter the cytosol. The mechanism of how Ca2+ ions triggers Ca2+ release from the SR was identified by several groups in the 1960s, and appropriately termed ‘ Ca2+-induced- Ca2+-release’ (Endo et al., 1968; Ford et al., 1968).

An overall increase in intracellular Ca2+ level causes Ca2+ to bind and cause a conformational change in Troponin C, a protein present on actin filaments. This conformational change causes a displacement of Tropomyosin, which prevents the interaction of myosin protein with actin filaments, thereby allowing myosin to contact actin, which promotes sarcomeric contraction. Alternatively, Ca2+ sequestration from myofilaments and cytoplasmic depletion prompts a relaxation of the sarcomere. Such a decrease of cytoplasmic Ca2+ occurs by either by re-entering the lumen of organelles, such as the SR and mitochondria, or cellular export by Ca2+ pumps and Na+/ Ca2+ exchangers on the sarcolemma (D. Bers, 2002).

The efficiency of muscle contraction is partly depicted by the type of myosin heavy chain (MyHC) that the cell expresses. MyHC are enzymes, found on the head of myosin proteins, which catalyze the hydrolysis of ATP. The rate at which MyHC can hydrolyze ATP ultimately depicts the speed at which the myofilaments contract, as well as the overall energy efficiency of that cell. In cardiomyocytes, two types of MyHC proteins are expressed: α-MyHC and β-

MyHC. The following table represents the distinguishing features of the cells that express either α-MyHC or β-MyHC:

Similar to skeletal muscles, cardiomyocytes are categorized into two distinct classes, based on the type of myosin heavy chain (MyHC) that is expressed. Those who predominantly express α-MyHC are found in adult hearts, contract in a more energy inefficient manner and are quicker to fatigue. In contrast, cardiomyocytes that express more β-MyHC are present in developing hearts, have a more energy efficient contraction and are more resistant to fatigue.

Cardiovascular diseases and pathological cardiac hypertrophy

Cardiovascular diseases are disorders that prevent the proper function of the heart and blood vessels, causing abnormalities of the cardiovascular system, which lead to defects in the brain, kidneys, lungs and other parts of the body (Public Health Agency of Canada, 2009). According to the World Health Organization, cardiovascular diseases accounted for 29% of global deaths in 2004, making it the leading cause of death in the world (World Health Organization, 2009). Furthermore, with an aging population, the number of patients diagnosed with heart disease in America is expected to double within the next 30 years, from 5 million to 10 million (Hobbs, 2004). In Canada, this disease was responsible for 31% (or > 70, 000) of total deaths in 2005 (Statistics Canada, 2009).

Amongst the numerous categories of cardiovascular diseases, heart failure is the most prevalent, with the fastest spreading rate and the highest mortality rate over the past decade (Heineke et al., 2006). Heart failure is defined by

defects in ‘ cardiomyocyte structure, function, rhythm or conduction’, which prevents the heart to pump adequate amounts of oxygenated blood and nutrients to meet the body’s demands (McMurray et al., 2005). Individuals living with a failing heart suffer from severe coughing, shortness of breath and edema, leading to a decreased tolerance to exercise and an overall diminishment in physical and mental health. As the disease progresses, patients may develop further pathophysiologies due to detrimental effects on the function of vital organs, ultimately resulting in death.

A common abnormality that precedes heart failure is the pathological enlargement of the heart, a condition known as cardiac hypertrophy. Cardiac hypertrophy is induced by the release of hormones, cytokines, chemokines and peptide growth factors, which act on cardiomyocytes in an endocrine, paracrine and autocrine manner (Heineke et al., 2006). The release of these factors occurs in response to increased cardiac workload, myocardial injury or defects in the contractibility of cardiomyocytes (J. Molkentin, 2000). The initial stage leading to cardiac hypertrophy is increased size and cell volume of cardiomyocytes in order to sustain the increased cardiac output demanded by the hypertrophied heart. Such a process is referred to as ‘ compensatory hypertrophy’. At later stages of cardiac hypertrophy, the hypertrophied heart can no longer keep up with the increased workload, which subjects patients to heart failure, cardiac arrhythmias and sudden death (Berenji et al., 2005).

It should be noted physiological cardiac hypertrophy, which occurs during pregnancy, adolescence growth and aerobic training, does not share the same detrimental consequences on cardiomyocytes as pathophysiological

heart growth (Oakley, 2001). A characteristic of pathologically hypertrophied hearts is cardiomyocyte disarray, which is a disorder of heart cells. Misaligned cardiomyocytes prompts a disruption in the conduction of action potentials across cells, leading to compromised intracellular Ca2+ kinetics and decreased shortening of the sarcomere, which ultimately compromises the contractions of the heart. The molecular signaling pathways, responsible for cardiac hypertrophy, are being extensively studied by researchers with the hopes of developing therapies to treat cardiac hypertrophy.

Calcineurin-NFAT signaling pathway

The availability of intracellular calcium (Ca2+) in mammalian cells is critical for their existence and proper function. In addition to its role in muscle cell electrophysiology and contraction, Ca2+ acts as a secondary messenger in many signal transduction pathways, involved in physiological processes such as fertilization, memory, apoptosis, membrane trafficking and cell division (D. M. Bers, 2008). Furthermore, at the molecular level, Ca2+ has been implicated in regulation of gene transcription, DNA replication, DNA repair and both protein synthesis and degradation.

A common question in muscle cell biology is that, with its numerous downstream targets, how does Ca2+ specify and activate a particular signaling pathway. It is generally understood that Ca2+ influxes into the cytoplasm through Ca2+ transporters on the sarcolemma as waves of Ca2+. In the 1990s, researchers have identified that depending on the amplitude and frequency at which Ca2+ waves penetrate the cell, different Ca2+-dependent signaling pathways are activated, which also affects gene expression and cell differentiation (Berridge, 1997; Dolmetsch et al., 1997;

Dolmetsch et al., 1998). However, the exact molecular mechanisms in which specific Ca2+-dependent pathways in contracting cardiomyocytes are regulated remains disputed due to the highly specialized rhythmic cycling of Ca2+ involved in the heart's ECC. Molkentin's group have postulated the existence of Ca2+ microdomains in the cytoplasm, which are relatively independent of the Ca2+ involved in the ECC. Within these microdomains, Ca2+ is locally regulated and can activate protein signaling pathways in that particular region (Houser et al., 2008).

Many proteins that require Ca2+ to be active cannot readily bind Ca2+, thus use Calmodulin (CaM), a high affinity Ca2+-binding protein, as a Ca2+ sensor and signal transducer. Expressed in all eukaryotic cells, CaM is a 17kDa protein composed of four EF-hand motifs, each capable of binding a single Ca2+ ion. The affinity to which Ca2+ binds CaM depends on changes in intracellular Ca2+ concentrations. When cytoplasmic Ca2+ level are low, CaM exists in a closed conformation, where the EF-hand motifs are packed together, hiding the Ca2+ binding sites. Alternatively, when intracellular Ca2+ level are high, Ca2+ ions bind to the EF hand motifs on CaM, causing a conformational change that allow Ca2+ to bind more readily to the other motifs, allowing CaM to attain an open configuration (Chin et al., 2000). Because CaM is a small, flexible molecule with numerous targets, such conformational changes are required to expose specific hydrophobic regions on each domain, which allow the Ca2+-CaM complex to bind and activate specific proteins (Al-Shanti et al., 2009).

One of the most recognized signaling pathways that require the Ca2+-CaM complex to be activated is the Calcineurin – Nuclear Factor of Activated T-Cells cascade.

Calcineurin (Cn), also referred to as protein phosphatase 2B (PP2B), is a Ca2+-dependent serine/threonine phosphatase that was first discovered in 1979 as a CaM binding protein in brain extracts (Klee et al., 1979). Further research by Schreiber's group identified that Cn played a prominent role in the immune system, where the addition of immunosuppressive drugs, cyclosporine A (CsA) and FK506, decreased Cn's activity (Liu et al., 1991). Cn is ubiquitously expressed in all cells and the gene that encodes the Cn protein is conserved from yeast to mammals, suggesting a common mode of regulation (Al-Shanti et al., 2009; Rusnak et al., 2000).

Once active, Cn can de-phosphorylate a number of transcription factors such as myocyte enhancer factor 2 (MEF2), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and nuclear factor of activated T-cells (NFAT) (Alzuherri et al., 2003; Blaeser et al., 2000; Jain et al., 1993; Michel et al., 2004). In addition to transcription factors, Cn has been identified as a direct regulator of the pro-apoptotic factor, Bcl-2 (Wang et al., 1999). The most characterized downstream target of Cn is the family of NFAT transcription factors. In the heart, the role of the Cn-NFAT signaling pathway in mediating pathological cardiac hypertrophy in vitro and in vivo has been extensively studied (Bueno et al., 2002; De Windt et al., 2001; Hill et al., 2002; Molkentin et al., 1998; Sussman et al., 1998; Zou et al., 2001). Once de-phosphorylated, NFAT transcription factors translocate to the nucleus and

dimerize with other transcription factors to re-activate cardiac fetal genes, leading to hypertrophy of the adult heart.

The structure of Calcineurin

Human Cn was first crystallized in 1995 by the Villafranca group (Kissinger et al., 1995). Although it shares similar sequence homology to other serine/threonine protein phosphatases, PP1 and PP2A, the structure of Cn was found to be unque due to its dependence on Ca2+ for optimal activity (Griffith et al., 1995; Kincaid et al., 1988; Klee et al., 1988). From its structure, it was discovered that Cn exists as a heterodimeric protein, consisting of two subunits: the 59kDa catalytic subunit, calcineurin A (CnA), and the 19kDa regulatory subunit, calcineurin B (CnB) (Kissinger et al., 1995).

The structure of CnA consists of two domains: a catalytic region which is found on the N-terminal and the regulatory domain which is present on the C-terminal region (Al-Shanti et al., 2009). The regulatory domain of CnA consists of three sub-domains: a CnB binding domain), a CaM binding domain) and an autoinhibitory domain (AI) as depicted in Figure 1. 4 (Ke et al., 2003; Klee et al., 1998). Alternatively, the structure of CnB shares a 35% sequence identity to CaM and contains four EF-hand motifs, allowing it to bind Ca2+ ions in a similar mechanism as CaM (Klee et al., 1988; Kretsinger et al., 1973).

In non-stimulated muscle cells, Cn is present in its inactive conformation, in the cytoplasm, where the autoinhibitory domain sterically blocks CnA’s catalytic domain, rendering the phosphatase inactive. Upon stimulation,

cytoplasmic Ca2+ will bind CnB, causing a conformational change, which exposes the CaM binding domain on CnA. Once the Ca2+-CaM complex docks onto its respective binding domain, another conformation change occurs which displaces the autoinhibitory domain from the catalytic domain, enabling the enzyme to be active.

The crystal structure of full length human Cn was solved with a resolution of 2. 1Çº. The globular structure of CnA consists of 521 residues, where residues 14-342 form the catalytic domain and residues 343-373 form the CnB binding helical domain (Kissinger et al., 1995). Residues 374-468 and 487-521 are not visible in the crystal structure because they are presumed to exist in a random conformation(Ke et al., 2003).

The AI domain is represented by a segment of 18 residues (Ser469-Arg486) that lie over the substrate-binding cleft on the C-terminus of CnA. The AI domain consists of two conserved short α-helical domains, with five additional residues in its extended form. The residues of the AI domain that have the strongest interactions with the substrate-binding cleft of CnA were identified as Glu481-Arg-Met-Pro484, where Glu481 hydrogen-bonds with water molecules bound to the dimetal site in Cn’s active site (Kissinger et al., 1995).

Residues 343-373 form an extended amphipathic α-helical region that interacts with hydrophobic residues within the CnB binding cleft.

In mammals, CnA is encoded by three genes (CnAα, CnAβ, CnAγ) and CnB by two genes (CnB1, CnB2). Yet in the heart, only CnAα, CnAβ and CnB1 are expressed (J. Molkentin, 2000).

NFAT proteins

NFAT transcription factors were first identified by the Crabtree group where, similar to Cn, NFAT played an important role in the regulation of early T-cell activation genes (Shaw et al., 1988). Since its discovery, researchers have provided evidence that the role of NFAT proteins was not restricted to T-cells, having been implicated in the ‘ central nervous system, blood vessels, heart, kidney, bone, skeletal muscle and haematopoietic stem cells’ (Crabtree et al., 2002; Graef et al., 2001; Hogan et al., 2003; Kiani et al., 2004; Macian, 2005).

NFAT proteins are part of the Rel-family of transcription factors. The molecular mass of NFAT ranges from 70-200kDa, which is due to alternative splicing of genes resulting in varying protein sizes and differential phosphorylation states (van Rooij et al., 2002). The primary structure of NFAT consists of a moderately conserved N-homology region (NHR), a conserved Rel-homology region (RHR) and a non-conserved C-terminal domain (CTD).

Firstly, the NHR (residues 1-407) contains a transactivation domain (TAD), a Cn docking site, a nuclear localization signal (NLS), a nuclear export signal (NES), serine-rich regions (SRR) and Ser-Pro-X-X-repeating motifs (SP), where X denotes any amino acid. The TAD is required for NFAT to bind the promoter region of genes to initiate transcriptional events. The Cn docking domain contains a SPRIEIT sequence, a variant of PxIxIT, which allows Cn to bind to NFAT and de-phosphorylate serine residues, mediating the nuclear shuttling of NFAT.

Secondly, the RHR (residues 408-677), which is conserved among all Rel proteins, confers to a shared DNA binding specificity (L. Chen et al., 1998). The C-terminus of the RHR contains a DNA binding motif, which permit Rel-proteins to bind the 5′-GGAAA-3′ consensus sequence (Rao, 1994). The N-terminus of the RHR contains a domain that allows NFAT to interact with other transcription factors in the nucleus. Such molecular partners include the leucine zipper protein activator protein-1 (Fos, Jun), the Zn-finger protein GATA-4, the MADS box protein MEF2 and many others (L. Chen et al., 1998; Crabtree et al., 2002; Hogan et al., 2003; Molkentin et al., 1998).

Lastly, although the exact role of the CTD (residues 678-928) remains ill defined, due to the differences in the length of the CTD between NFAT isoforms, it is possible that the CTD is responsible for the different transcriptional activity of the NFAT isoforms, as shown by several groups (Calabria et al., 2009; Rinne et al., 2010).

NFAT transcription factors are ubiquitously expressed and consists of five isoforms: NFATc1, NFATc2, NFATc3, NFATc4 and NFAT5 (also known as tonicity-responsive enhancer-binding protein or TonEBP) (Mancini et al., 2009). Of the five NFAT proteins, only NFATc1, NFATc2, NFATc3 and NFATc4 are regulated by Ca2+-Cn signaling and are have known roles in skeletal and cardiac muscles (Calabria et al., 2009; van Rooij et al., 2002). NFAT5 cannot interact with Cn due to the absence of a SPRIEIT domain and is therefore insensitive to Ca2+-Cn signaling (Lopez-Rodriguez et al., 1999). Rather, NFAT5 is regulated by osmotic stress and is known to control the expression of cytokines, such as tumor-necrosis factor (TNF) and lymphotoxin-β, in lymphocytes (Lopez-Rodriguez et al., 2001; Macian, 2005). Due to its

insensitivity of Cn and unclear roles in muscle cells, for the remainder of this thesis, the focus will be on the Ca2+-Cn regulated NFAT isoforms: NFATc1, NFATc2, NFATc3 and NFATc4.

The cellular localization of NFAT proteins depend on the phosphorylation state of approximately 14 serine residues on the NHR. Okamura et al. identified that of these residues, 13 phosphoserines are targeted by Cn and are located in motifs SRR1, SP2 and SP3 (Macian, 2005; Okamura et al., 2000). Upon de-phosphorylation, the NLS sequence of NFAT is exposed and the NES is masked, prompting nuclear entry. NFAT kinases are regulators of NFAT transcription factors, which can interact with NFAT and reversibly phosphorylate the same serine residues that are targeted by Cn. Known NFAT kinases include casein kinase-1 (CK-1), glycogen-synthase 3β (GSK3-β), p38 and JUN-N-terminal kinase (JNK) (Beals, Sheridan et al., 1997; Chow et al., 1997; Gomez del Arco et al., 2000; Zhu et al., 1998). Upon re-phosphorylation, the NES sequence is re-exposed whereas the NLS sequence is hidden, prompting cytoplasmic retention of NFAT (Okamura et al., 2000). These kinases can either be classified as maintenance kinases, which phosphorylate NFAT in the cytosol to prevent nuclear import or export kinases, which target NFAT in the nucleus to promote nuclear export. Each kinase can phosphorylates serine residues on specific motifs. CK-1 acts as both an export and maintenance kinase on SRR1 of NFATc2 (Okamura et al., 2004). GSK3-β functions as an export kinase on both SP2 and SP3 of NFATc1 and SP2 on NFATc2 (Beals, Clipstone et al., 1997; Macian, 2005). The mitogen activated protein kinase (MAPK) family consists of p38, JNK and extracellular-regulate-signal kinases (ERK) and can phosphorylate the first

serine of SRR1 on different NFAT isoforms. JNK phosphorylates NFATc1, whereas p38 targets NFATc2 (Chow et al., 1997; Gomez del Arco et al., 2000). CK1 may be responsible for phosphorylating the remaining serines on SRR1 (Macian, 2005). Although a cell may have the potential to translate different NFAT isoforms, depending on which NFAT kinase is expressed, only certain NFATs may be nuclear localized.

Cn-NFAT signaling in cardiac hypertrophy

Cn-NFAT signaling is described as a multifunctional regulator, where its function depends on the cell type in which this pathway is active. In the brain, Cn-NFAT signaling mediates numerous processes, which include memory, brain strokes, ischemic injury, Parkinson and Alzheimer's disease and the regulation of the cAMP-response-element binding protein (CREB) (Shibasaki et al., 2002). In the lungs, Cn-NFAT signaling has been implicated in the perinatal lung maturation and function, and regulating genes involved in the homeostasis of pulmonary surfactant, which is required for proper breathing (Dave et al., 2006). In skeletal muscles, this pathway is required for functional-overload induced skeletal muscle hypertrophy and for mediating skeletal muscle-fiber type conversions from fast muscle fiber type to slow muscle fiber type (Dunn et al., 1999; Michel et al., 2004). In the cardiovascular system, Cn is required for the early development of the heart, specifically the cardiac septum and valves (de la Pompa et al., 1998; Ranger et al., 1998). During heart disease, Cn-NFAT signaling promotes the reactivation of cardiac fetal genes, which are responsible for cardiac growth during development. The reactivation of these genes in the adult heart is

responsible for the pathological growth of the heart, and not physiological growth (Wilkins et al., 2004).

In 1998, Molkentin et al. first reported the novel role that Cn-NFAT signaling played in mediating pathological cardiac hypertrophy (Molkentin et al., 1998). Among the major findings of this report was that Cn-induced the de-phosphorylation of NFATc4, prompting its nuclear entry and allowed NFATc4 to interact with the GATA-4 transcription factor, leading to cardiac hypertrophy. In addition, cultured cardiomyocytes, treated with Cn inhibitors CsA and FK-506 immunosuppressive drugs, blocked chemical-induced cardiac hypertrophy. To support their in vitro findings, transgenic mice that expressed a cardiac-specific constitutively active form of CnA were generated. The hearts of CnA overexpressing transgenic mice, compared to the hearts of wild-type counterparts, displayed a 2-to-3 fold increase in heart weight-to-body weight ratio, a thickening of the left ventricular wall and intraventicular septum, a 2-fold increase in cross-sectional area of cardiomyocytes and extensive fibrosis. Furthermore, CnA overexpressing mice had a greater increased susceptibility to sudden death, mimicking the effects of heart failure in humans. Upon treatment with the Cn inhibitor, CsA, the hearts of CnA transgenic mice returned to normal size.

Many genes and proteins that are re-employed in response to heart disease have prominent functions in embryonic and fetal heart development. For example, cardiac fetal genes are active during the physiological growth in developing hearts. This family of genes consists of atrial natriuretic factor (ANF), b-type natriuretic peptide (BNP), α-myosin heavy chain (α-MHC), β-myosin heavy chain (β-MHC), and many others (Oka et al., 2007). When the

heart has fully matured into an adult heart, the expression of these genes becomes dormant. During heart disease, hypertrophic stimuli re-activate the expression of these genes in the adult heart, which enables the heart to grow to a pathological state.

One of the most studied transcription factor that interacts with NFAT to initiate cardiac hypertrophy are GATA proteins. GATA transcription factors consist of two conserved zinc fingers that are required to bind to the consensus DNA sequence 5′-(A/T)GATA(A/G)-3′, as well as domains that allow GATA to interact with transcriptional cofactors (Ko et al., 1993; Merika et al., 1993; Oka et al., 2007). Of the six members of the GATA family (GATA-1 to GATA-6), GATA-4, GATA-5 and GATA-6 are expressed in the heart (J. D. Molkentin, 2000). Among the GATA proteins expressed in the heart, GATA-4 has been associated with embryonic cardiogenesis, such as heart tube formation, and pathological growth of the adult heart (Molkentin et al., 1997; Pikkarainen et al., 2004). In addition, GATA-4 is a known regulator of the expression of cardiac structural genes during development.

GATA-4 gene targeted mice were embryonic lethal at E7-9. 5 due to structural and functional defects of the heart (Molkentin et al., 1997). Alternatively, cultured cardiomyocytes overexpression of GATA-4 caused a 2-fold increase in cell surface area, whereas GATA-4 overexpressing transgenic mice lead to increased heart-weight-to-body weight ratio, cardiomyopathy features of the cells and upregulation in the expression of cardiac fetal genes (Liang, De Windt et al., 2001).

The regulation of GATA-4 occurs post-translationally, where such modifications affect its DNA binding ability, transcriptional activity and cellular localization. A number of chemical stimuli that induce cardiac hypertrophy have been associated with the phosphorylation of GATA-4, which increases both its DNA binding and transcriptional activity (Oka et al., 2007; Pikkarainen et al., 2004). Molkentin's group identified that phosphorylation of Ser105 on GATA-4 by the ERK1/2 and p38 MAPK was responsible for GATA-4 increased DNA binding affinity and transactivation during heart failure (Charron et al., 2001; Liang, Wiese et al., 2001). Another kinase that targets GATA-4 is GSK3-β, a known negative regulator of cardiac hypertrophy (Haq et al., 2000). GSK3-β-mediated phosphorylation of GATA-4 prompts its export from the nucleus, rescuing Cn-mediated cardiac hypertrophy (Morisco et al., 2001).

A second family of transcription factor that is re-activated during heart disease is the myocyte enhancer factor 2 (MEF2). There are four members of the MEF2 family expressed in vertebrates: MEF2A, MEF2B, MEF2C and MEF2D. MEF2 proteins can either homodimerize or heterodimerize with other transcription factors such as NFAT and GATA, which can then bind to the DNA sequence 5′-CTA(A/T)4TAG-3′ to carry out transcriptional events (Blaeser et al., 2000; McKinsey et al., 2002; Morin et al., 2000; Oka et al., 2007). Although the MEF2 proteins are expressed in most cell types, their transcriptional activity is restricted to the immune system, neurons and contractile muscle cells (Akazawa et al., 2003).

In the heart, MEF2 have critical roles in cardiac differentiation. MEF2C null mice were embryonic lethal, due to cardiac looping defects, an absence of a

right ventricle and a downregulation of cardiac structural genes (Bi et al., 1999; Lin et al., 1997; Oka et al., 2007). The majority of MEF2A null mice died 2-10 days after birth because of defects in conduction and architecture of the heart. Surviving MEF2A null mice displayed reduced mitochondrial content and a less efficient conductive system. (Naya et al., 2002). In addition, transgenic mice that express a dominant negative MEF2 died shortly after birth because of cardiomyocyte hypoplasia, thinning of the ventricular walls and heart chamber dilation (Kolodziejczyk et al., 1999; Oka et al., 2007).

A greater workload imposed on the heart, a phenotype of cardiac hypertrophy, has been associated with increased MEF2-DNA binding (Molkentin et al., 1993; Nadruz et al., 2003). In cultured cardiomyocytes, adenoviral-mediated overexpression of MEF2A or MEF2C caused sarcomeric degeneration and cell elongation, both of which indicate cardiac dilatation. The hearts of transgenic mice overexpressing MEF2A or MEF2C were subject to contractile defects, ventricular dilation and were more readily hypertrophied when pressure overload stimulation was induced. However, when cells of the transgenic hearts were isolated, rather than having a greater cross-sectional area, the cardiomyocytes were more elliptical in shape, suggesting that MEF2 did not d