
Reactive Oxygen Species Signaling in Cardiac Remodeling and Failure

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Abstract

Despite major advances in treatment, chronic heart failure (CHF) remains one of the leading causes of mortality and morbidity in the western world. Although the stimuli for CHF are wide ranging, the underlying pathophysiological response, known as cardiac remodeling, is broadly similar. The process of cardiac remodeling is characterized by a series of initially adaptive structural and functional alterations which become progressively maladaptive and ultimately lead to CHF. Oxidative stress and reactive oxygen species (ROS) play a well-established role in the development and progression of cardiac remodeling. ROS include both free radicals (e.g., superoxide, hydroxyl) and oxidants (e.g., peroxynitrite, hydrogen peroxide) and exert important physiological actions, in addition to their well-documented harmful effects. Increased ROS production has been widely reported in experimental and clinical CHF and has been linked to several key remodeling processes such as cardiomyocyte hypertrophy, apoptosis, contractile dysfunction, and extracellular matrix remodeling. It is now apparent that myocardial ROS function as key regulators of intracellular signaling that are capable of altering the activity of numerous molecules and pathways to exert subtle modulatory effects on cardiac remodeling. Indeed, several key signaling pathways involved in CHF pathogenesis, including MAPKs, Akt, NF- κ B, and AP-1 are known to be redox sensitive, with varying thresholds for activation. The downstream effects of ROS appear to be critically dependent upon a number of factors including the source of ROS, cellular localization, temporal regulation, and stimulus for production, which dictate the highly specific and targeted phenotypic changes associated with cardiac remodeling. Although the precise mechanisms underlying ROS-dependent modulation of myocardial signaling remain unknown, it is plainly evident that a more complete delineation of these pathways in cardiac remodeling and CHF holds clear potential for the identification of improved therapeutic targets.

Keywords

Cardiomyocyte hypertrophy • Chronic heart failure • Extracellular matrix remodeling • Mitochondria • NADPH oxidases • Reactive oxygen species • Serine/threonine protein kinases • Transcription factors

Introduction

Chronic heart failure (CHF) affects up to 2 % of the adult population in the western world and is the leading cause of morbidity and mortality (Jessup and Brozena 2003). The incidence of CHF is rapidly increasing, and despite improved treatment with agents such as ACE inhibitors and β -blockers, it carries a poor prognosis, with reported 1-year mortality rates of up to 45 % (Jessup and Brozena 2003; Dolezal 2006). The causes of CHF are often multifactorial and include myocardial ischemia, hypertension, cardiotoxicity, valvular heart disease, and cardiomyopathy, although the underlying pathophysiological response, collectively known as cardiac

remodeling, is broadly similar (Swynghedauw 1999; Jessup and Brozena 2003). The process of cardiac remodeling is characterized by substantial alterations in myocardial structure, which are initially adaptive in maintaining or improving contractile function. However, progressive remodeling leads to increased wall stress, cardiac chamber dilatation, contractile dysfunction, and ultimately CHF (Brilla et al. 1990; Swynghedauw 1999). Initially, cardiac remodeling was considered to occur consequent of compensatory mechanisms producing direct toxic actions on the heart; however, accumulating evidence indicates that the process itself significantly contributes to disease progression in heart failure (Kurrelmeyer et al. 1998; Mann 1999). Despite improved clinical management of CHF with agents such as ACE inhibitors and β -adrenoceptor blockers, which have been shown to attenuate cardiac remodeling and confer morbidity/mortality benefits, there remains a substantial incidence of CHF even in optimally treated patients (Francis 2001). An improved and detailed understanding of the mechanisms underlying the pathogenesis of CHF is therefore essential in the search for novel therapeutic CHF targets.

Oxidative Stress and Cardiac Remodeling

It is now well established that oxidative stress and reactive oxygen species (ROS) play a key role in the development and progression of cardiac remodeling, with increased ROS production being widely reported in both experimental and clinical CHF (McMurray et al. 1993b; Hill and Singal 1996; Dieterich et al. 2000; Date et al. 2002; Heymes et al. 2003). At the cellular level, ROS have been linked to several key processes including cardiomyocyte hypertrophy, apoptosis, contractile dysfunction, extracellular matrix remodeling (ECM), inflammation, and vascular remodeling, each of which will be discussed in detail in this section.

Cardiomyocyte Hypertrophy

One of the most characteristic features of the remodeled heart is cardiomyocyte hypertrophy, which occurs in response to increased cardiac load. At the molecular level, stretching of the cardiomyocyte results in local neurohumoral activation, e.g., norepinephrine, angiotensin II (Ang II), endothelin-1 (ET-1), and significant alterations in gene expression which are largely responsible for the hypertrophic response (Swynghedauw 1999; Cohn et al. 2000). While it is thought to be initially adaptive by normalizing wall stress, progressive cardiomyocyte hypertrophy becomes maladaptive resulting in increased wall stress, ventricular dilatation, contractile dysfunction, and eventually development of CHF (Brilla et al. 1990; Swynghedauw 1999). Indeed, left ventricular hypertrophy (LVH) itself is a major independent risk factor for ischemic CHF and sudden death and is one of the most potent predictors of adverse cardiovascular outcomes in hypertensive patients (Gradman and Alfayoumi 2006). Over recent years, oxidative stress has become

inexorably linked with the development and progression of cardiomyocyte hypertrophy. For example, increased ROS production has been widely reported in both experimental and clinical LVH (Treasure et al. 1993; Dhalla et al. 1996; Date et al. 2002; Yasunari et al. 2004). Increased ROS production is also implicated in the hypertrophic response of isolated cardiomyocytes induced by various stimuli including norepinephrine, Ang II, ET-1, phenylephrine (PE), tumor necrosis factor- α (TNF- α), and cyclic stretch (Nakamura et al. 1998; Pimentel et al. 2001; Hirotani et al. 2002). Furthermore, inhibition of the endogenous antioxidants, Cu-Zn superoxide dismutase (SOD) and thioredoxin, also induces oxidative stress and cardiomyocyte hypertrophy in vitro and in vivo, respectively (Siwik et al. 1999; Yamamoto et al. 2003). Similarly, the development and progression of experimental pressure-overload LVH was inhibited by either treatment with the antioxidants, vitamin E, N-acetylcysteine, and hemoxygenase-1, or overexpression of endogenous catalase or thioredoxin-2, supporting a role for ROS production in cardiac hypertrophy in vivo (Dhalla et al. 1996; Byrne et al. 2003; Hu et al. 2004; Widder et al. 2009; Qin et al. 2010).

Extracellular Matrix Remodeling

One of the defining characteristics of cardiac remodeling is a marked increase in interstitial fibrosis, which is thought to be largely responsible for increased myocardial stiffness and the development of diastolic dysfunction in CHF (Litwin and Grossman 1993; Weber et al. 1994; Swynghedauw 1999). The process of ECM remodeling involves a complex reorganization and delicate balance between collagen deposition and degradation (Swynghedauw 1999). The main driving force for matrix turnover is a family of zinc-containing endoproteases, known as matrix metalloproteinases (MMPs), whose activity and expression is markedly altered in CHF (Spinale et al. 2000; Creemers et al. 2001). The activity of MMPs is controlled by TIMPs (tissue inhibitors of MMPs), which are also dysregulated in CHF and work in tandem to control collagen deposition and accumulation (Li et al. 1998; Sun and Weber 2000). MMP activation not only facilitates the initial development of fibrosis but is also implicated in subsequent left ventricular (LV) dilatation which occurs during the transition to CHF (Peterson et al. 2001; Spinale 2002). The key role of MMPs in cardiac remodeling is underlined by studies in which pharmacological inhibition or genetic manipulation was found to attenuate LV dilatation and contractile dysfunction in experimental CHF (Matsusaka et al. 2006; Morita et al. 2006). The process of ECM remodeling is known to be particularly redox sensitive, with key underlying processes, such as fibroblast proliferation, collagen synthesis, and MMP activation/expression, being modulated by ROS (Kunsch and Medford 1999; Siwik et al. 2001; Zhang et al. 2002). Indeed, several of the main pathophysiological stimuli that drive interstitial fibrosis and matrix turnover, such as Ang II, cytokines, and cyclic load, also stimulate intracellular ROS production (Kunsch and Medford 1999; Sano et al. 2001). Furthermore, the development of cardiac interstitial fibrosis driven specifically by Ang II or aldosterone and associated MMP

activation have been demonstrated to be mediated by ROS (Bendall et al. 2002; Sun et al. 2002; Johar et al. 2006). Chronic treatment with the antioxidants dimethylthiourea, probucol, and apocynin is also reported to attenuate the progression of cardiac fibrosis in vivo, confirming an important role for ROS in this setting (Kinugawa et al. 2000; Sia et al. 2002; Park et al. 2004).

Contractile Dysfunction

Structural remodeling of the cardiomyocyte and extracellular matrix is known to have a significant impact on cardiac contractile function. However, the development and progression of CHF is also characterized by marked effects on cardiomyocyte biology, such as alterations in membrane protein expression, excitation-contraction coupling, myofilament calcium sensitivity, and organization of the cytoskeleton, together with significant changes in the extracellular matrix (as discussed above), all of which contribute to the onset of contractile dysfunction and subsequent transition to CHF (Swynghedauw 1999). It is well established that contractile dysfunction in CHF is associated with oxidative stress and increased ROS production (McMurray et al. 1993a; Mallat et al. 1998; MacCarthy et al. 2001; Byrne et al. 2003). Indeed, in a murine model of pressure overload, treatment with the antioxidants, N-acetylcysteine and tempol, reduced ROS production and cardiac contractile dysfunction, whereas global deficiency of extracellular SOD exacerbated myocardial oxidative stress and contractile dysfunction (Grieve et al. 2006; Chess et al. 2008; Lu et al. 2008). In isolated cardiomyocytes, elevated ROS levels have been shown to suppress contractile function which is restored by antioxidants (Ye et al. 2004; Dong et al. 2006; Li et al. 2008; Rueckschloss et al. 2010). At the cellular level, ROS have been reported to impair calcium transients, modulate opening of ryanodine receptors, decrease L-type calcium channel current, and inhibit activity of the sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA), all of which are important regulators of cardiomyocyte contractility (Gill et al. 1995; Xu et al. 1997; Kawakami and Okabe 1998; Zima et al. 2004; Rueckschloss et al. 2010). The precise mechanisms by which ROS modulate Ca^{2+} handling and contractile function in cardiomyocytes are unknown. However, recent studies suggest an important role for oxidative protein modifications of thiols on SERCA, the sodium- Ca^{2+} exchanger, and ryanodine receptor 2 (Belevych et al. 2007; Lancel et al. 2010; Kuster et al. 2010).

Cardiomyocyte Apoptosis

The major cause of cardiomyocyte death during cardiac remodeling is apoptosis, which not only results in significant loss of contractile tissue, but also stimulates compensatory cardiomyocyte hypertrophy and reparative fibrosis, all of which contribute to associated contractile dysfunction (Swynghedauw 1999). Apoptosis is a highly regulated form of programmed cell death occurring by two main

pathways, both of which converge on caspase-3 activation resulting in nuclear degradation and characteristic DNA fragmentation (van Empel et al. 2005; Anilkumar et al. 2009). The intrinsic mitochondrial pathway involves opening of the mitochondrial permeability transition pore (MPTP), typically in response to stimuli such as ischemia and hypoxia, resulting in the release of several proteins, including cytochrome c, which complexes with caspase-9, triggering activation of caspase-3. The extrinsic pathway is stimulated by binding of specific ligands, such as Fas or TNF- α , to death receptors resulting in formation of the death-inducing signaling complex (DISC) and subsequent activation of caspase-8 and caspase-3 (van Empel et al. 2005). Although cardiomyocyte apoptosis is widely reported to occur at very low levels in both experimental and clinical CHF, it is thought to make a key contribution to the development and progression of cardiac remodeling (Kang and Izumo 2000). This is exquisitely demonstrated by a recent study in which conditional cardiac-specific activation of caspase-8 was found to result in severe dilated cardiomyopathy, despite only 0.23 % of the cardiomyocytes undergoing apoptosis (Wencker et al. 2003). It is well established that ROS can stimulate cardiomyocyte apoptosis (von Harsdorf et al. 1999). For example, oxidative stress has been continually associated with cardiomyocyte apoptosis occurring in response to several in vivo stimuli including ischemia, pressure overload, and cardiotoxicity (Looi et al. 2008; El Azzouzi et al. 2010; Zhao et al. 2010). Furthermore, hydrogen peroxide (H₂O₂) causes cardiomyocyte apoptosis both in vitro and in vivo, and the process has been shown to be sensitive to treatment with antioxidants (Aoki et al. 2002; Kwon et al. 2003; Qin et al. 2006; Qin et al. 2007, 2010). In addition to directly initiating apoptosis, ROS have been reported to exert several other important actions including mediation of apoptotic signaling, modulation of antiapoptotic systems, transcriptional regulation of apoptosis-relevant genes, and causation of direct mitochondrial damage (Anilkumar et al. 2009).

Inflammation

Over recent years, inflammation has emerged as an increasingly important player in the cardiac remodeling process (Heymans et al. 2009; Oikonomou et al. 2011). The first suggestion of the importance of inflammatory activation came from initial reports of raised plasma cytokine levels in CHF patients (Rauchhaus et al. 2000; Deswal et al. 2001). Although subsequent experimental studies have indicated that anti-inflammatory therapies may be beneficial in CHF (Zhou et al. 2006; Westermann et al. 2007), large-scale clinical trials of TNF- α blockade in this setting have provided disappointing results and may even be detrimental (Chung et al. 2003; Mann et al. 2004). Nonetheless, it is clear that increased inflammatory cell infiltration is a hallmark of CHF, which appears to play a key role in regulating underlying processes such as ECM remodeling and cardiomyocyte apoptosis (Zhou et al. 2006; Westermann et al. 2011). Although the underlying mechanisms remain unclear, it is evident that oxidative stress and increased ROS production are important in both mediating inflammatory cell activation and the ensuing

myocardial response in experimental and clinical CHF (Machida et al. 2003; Chiang et al. 2006; Mercurio et al. 2007; Gao et al. 2008; Zhao et al. 2010). Furthermore, cardiac inflammation and remodeling are inhibited by antioxidants, underlining a key role for ROS in this setting (Muller et al. 2000; Xu et al. 2011).

Vascular Remodeling

Coronary angiogenesis is tightly linked to cardiac growth during development and is considered to be an important component of the adaptive response to myocardial stress (Giordano et al. 2001; Hilfiker-Kleiner et al. 2004). Indeed, it is thought that an imbalance between vascular and myocardial remodeling may be a key factor in determining progression to CHF (Shiojima et al. 2005). Myocardial vascular endothelial growth factor (VEGF) production appears to be one of the key regulators of angiogenesis in CHF, which is itself controlled by transcription factors such as hypoxia-inducible factor-1 α (HIF-1 α) and GATA4 (Shiojima et al. 2005; Heineke et al. 2007; Sano et al. 2007). Interestingly, it now appears that expression and activity of these pro-angiogenic factors may be redox sensitive. For example, VEGF is known to stimulate ROS production which plays a key role in downstream pro-angiogenic signaling, which is also attenuated by antioxidant treatment (Colavitti et al. 2002; Ushio-Fukai et al. 2002). Furthermore, ROS can activate upstream HIF-1 α both directly and indirectly via phosphatase inhibition, thereby controlling activity of pro-angiogenic factors such as VEGF and erythropoietin (Kietzmann and Gollach 2005; Bonello et al. 2007). Of particular interest is a recent study demonstrating that cardiomyocyte-derived H₂O₂ specifically promotes increases in myocardial capillary density, thereby conferring protection against adverse cardiac remodeling and dysfunction in response to pressure overload (Zhang et al. 2010).

ROS as Signaling Molecules

It is well established that ROS production and oxidative stress play a key role in the pathophysiology of cardiac remodeling, with increased ROS levels widely reported in both experimental and clinical CHF (Beswick et al. 2001; MacCarthy et al. 2001; Landmesser et al. 2002; Heymes et al. 2003). ROS in the cardiovascular system include the free radicals, superoxide (O₂^{•-}) and hydroxyl ([•]OH), in addition to the powerful oxidants, peroxynitrite (ONOO⁻) and hydrogen peroxide (H₂O₂), and these are summarized in Fig. 40.1. Although the harmful cardiovascular effects of ROS are well documented, it is becoming increasingly apparent that ROS play a critical role in normal physiological processes, in particular as specific regulators of intracellular signaling pathways (Finkel 2011). For example, fertilization of a sea urchin egg results in a large increase in H₂O₂, which, rather than damaging the organism, serves to facilitate development of a protective embryonic matrix (Wong et al. 2004). In addition, inhibition of epidermal growth factor

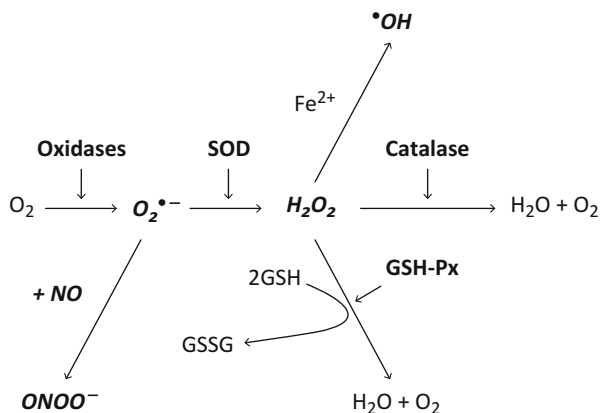


Fig. 40.1 Schematic representation of the major ROS-producing pathways in the cardiovascular system. Oxidases convert oxygen to superoxide ($O_2^{\bullet -}$), which may then be either dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) or rapidly react with nitric oxide (NO) to form highly toxic peroxynitrite ($ONOO^-$). H_2O_2 can then be further converted to (1) hydroxyl radical ($\bullet OH$) after reaction with Fe^{2+} or (2) H_2O and O_2 by catalase or glutathione peroxidase (GSH-Px) via the oxidation of glutathione (GSH) to glutathione disulphide (GSSG)

(EGF)-induced H_2O_2 production in A431 human epidermoid carcinoma cells resulted in marked attenuation of associated tyrosine phosphorylation of a range of key cellular proteins (Bae et al. 1997). Furthermore, downregulation of mitochondrial complex III in human fibroblasts prevented hypoxia-induced stabilization of HIF-1 α , suggesting a crucial role for mitochondrial ROS in the setting of oxygen sensing (Brunelle et al. 2005). In the heart, ROS signaling has been shown to play a key physiological function in both cardiac development via regulation of proliferation and differentiation (Sauer et al. 2000) and regulation of excitation-contraction coupling via redox modulation of the ryanodine receptor (Zima and Blatter 2006; Hidalgo and Donoso 2008; Santos et al. 2011). It is now widely recognized that ROS are capable of altering the activity of numerous intracellular molecules and signaling pathways to exert subtle modulatory effects that can induce highly specific phenotypic changes. In health, the production and activity of ROS are tightly regulated by several endogenous antioxidant systems, which serve to maintain physiological ROS levels sufficient for basal signaling. These antioxidant systems are both enzymatic and nonenzymatic in nature. Enzymatic antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase (Sies 1997). Superoxide dismutases, of which there are three isoforms (cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD), represent the major mechanism by which excess $O_2^{\bullet -}$ is degraded to H_2O_2 (Giordano 2005). Thioredoxin and thioredoxin reductase together form an additional enzymatic antioxidant and redox regulatory system that has been implicated in a wide variety

of ROS-related processes (Nordberg and Arner 2001). Thioredoxin and thioredoxin reductase can catalyze the regeneration of many antioxidant molecules, including ubiquinone (Q10), lipoic acid, and ascorbic acid, and as such constitute an important antioxidant defense mechanism. Examples of nonenzymatic antioxidants include vitamins C and E and β -carotene as well as glutathione, all of which work in combination to regulate ROS levels (Chen et al. 2012). In the normal physiological situation, ROS and endogenous antioxidants therefore act in concert to maintain normal signaling processes. However, in pathological conditions oxidative stress may occur due to an imbalance between ROS generation and antioxidant activity. Increased ROS levels then exert deleterious oxidative damage to DNA, proteins, lipids, membranes, and macromolecules, which underlie many of the changes associated with adverse cardiac remodeling (Cave et al. 2005). It is becoming increasingly clear that the source and location of cellular ROS production is critical in determining their downstream effects. Once thought of as nonspecific signaling molecules, it is now apparent that ROS signaling may be regulated in both a spatial and temporal manner to exert highly targeted effects which dictate distinct phenotypic changes. In this regard, important potential enzymatic sources of ROS in the cardiovascular system will now be discussed.

Cardiovascular Sources of ROS

Xanthine Oxidase

The xanthine oxidase enzyme is a form of xanthine oxidoreductase, which is interconvertible with xanthine dehydrogenase, and produces $O_2^{\bullet -}$ as a by-product of the terminal steps of purine metabolism. Specifically, xanthine oxidase catalyzes the single electron reduction of molecular oxygen to $O_2^{\bullet -}$ by promoting the conversion of hypoxanthine to xanthine and then uric acid. Basal levels of xanthine oxidase are low, although increases in expression and xanthine oxidase-mediated ROS production have been demonstrated in both experimental (Ekelund et al. 1999; Ukai et al. 2001) and clinical end-stage CHF (Leyva et al. 1998; Cappola et al. 2001). In addition, temporal alterations in xanthine oxidoreductase expression have been reported in rat models of left and right ventricular failure, suggesting that this enzyme system may be important in the transition from compensation to CHF (De Jong et al. 2000). Furthermore, several studies have documented beneficial effects of the xanthine oxidase inhibitor, allopurinol, in experimental models of pacing-induced CHF, dilated cardiomyopathy, and chronic myocardial infarction (MI), with reported improvements in LV contractile function, calcium cycling, myocardial efficiency, and adverse cardiac remodeling (Engberding et al. 2004; Stull et al. 2004; Duncan et al. 2005; Minhas et al. 2006; Saliaris et al. 2007). These effects appear to translate to the clinical setting as allopurinol treatment has been shown to result in acute improvement in myocardial efficiency and reduced mortality in CHF patients (Cappola et al. 2001; Wei et al. 2009) although larger scale clinical studies have reported limited benefits (Gavin and Struthers 2005; Pacher et al. 2006).

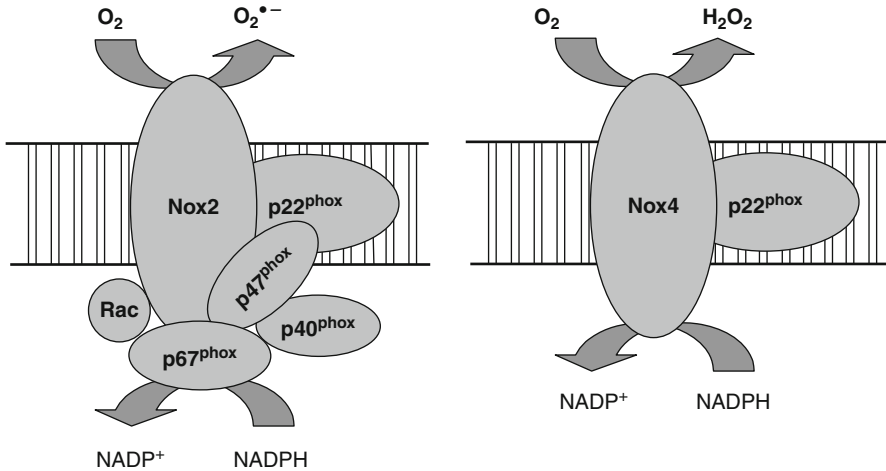


Fig. 40.2 Structure of cardiac NADPH oxidases. Nox2 and Nox4 NADPH oxidases are expressed throughout the heart. They both contain a membrane-bound core (Nox2 or Nox4 together with p22^{phox}) but have differing activities and functions. Nox2 NADPH oxidase activity requires the association of several regulatory cytosolic subunits (p47^{phox}, p67^{phox}, p40^{phox}, Rac) and is thought to generate mainly superoxide using NADPH as the electron donor. In contrast, Nox4 is constitutively active and is thought to primarily produce H₂O₂

NADPH Oxidases

NADPH oxidases are a family of ROS-generating enzymes which are established as a major source of cardiac O₂^{•-} production (Li et al. 2002; Byrne et al. 2003). The original NADPH oxidase was first described in neutrophils, where it produces high levels of ROS during phagocytosis (the “respiratory burst”) by catalyzing the single electron reduction of molecular oxygen, using NADPH as the electron donor (Griendling et al. 2000). Interestingly, unlike other sources, the primary function of NADPH oxidases appears to be ROS generation (Lambeth 2004). Seven NADPH oxidase family members have now been identified (Nox1-5 and Duox1-2), which are each based on a distinct catalytic subunit with tissue-specific expression (Lambeth. 2004; Brown and Griendling 2009). The major cardiac isoforms appear to be Nox2 and Nox4 (see Fig. 40.2), which are expressed in cardiomyocytes, fibroblasts, and endothelial cells (Anilkumar et al. 2008). The prototypic Nox2 NADPH oxidase is comprised of a membrane-bound catalytic core consisting of one Nox2 and one p22^{phox} subunit and several regulatory cytosolic subunits (p47^{phox}, p67^{phox}, p40^{phox}, and Rac1), which associate with the heterodimer upon activation by stimuli such as Ang II, ET-1, VEGF, and TNF- α (Griendling et al. 2000; Lambeth. 2004). In contrast, Nox4 NADPH oxidase is comprised of a single Nox4/p22^{phox} heterodimer localized to the perinuclear endoplasmic reticulum and mitochondria and is constitutively active, being mainly

regulated by protein level (Block et al. 2009; Brown and Griendling 2009). Interestingly, emerging data indicates that Nox2 NADPH oxidase may produce primarily $O_2^{\cdot-}$, whereas Nox4 generates mainly H_2O_2 (Martyn et al. 2006; Bedard and Krause 2007; Dikalov et al. 2008; Takac et al. 2011). Importantly, H_2O_2 is much more stable than $O_2^{\cdot-}$ and can diffuse widely, which may account for distinct effects of different ROS forms on cellular redox regulation and underlie the apparent complexity of Nox signaling. Under physiological conditions, Nox NADPH oxidases generate low levels of ROS which are thought to be involved in basal intracellular signaling (Brown and Griendling 2009). However, it is well established that under pathological conditions, myocardial NADPH oxidase activity and expression is markedly increased and contributes significantly to CHF development and progression (Byrne et al. 2003; Heymes et al. 2003; Looi et al. 2008; Zhao et al. 2010). Specifically, NADPH oxidase-derived ROS have been shown to be critically involved in several of the cellular processes involved in cardiac remodeling, including interstitial fibrosis, MMP activation, cardiomyocyte hypertrophy, contractile dysfunction, and apoptosis (MacCarthy et al. 2001; Sun et al. 2002; Park et al. 2004; Rude et al. 2005; Qin et al. 2006). Interestingly, recent studies suggest that Nox2 and Nox4 NADPH oxidases exert differential effects on the heart which are dependent on the stimulus for remodeling. Studies in Nox2-deficient mice found that Nox2 NADPH oxidase was critical for cardiomyocyte remodeling and interstitial fibrosis induced by MI, Ang II, and doxorubicin (Bendall et al. 2002; Looi et al. 2008; Zhao et al. 2010). In contrast, cardiac hypertrophy in response to chronic pressure overload was unaltered in Nox2-deficient mice which showed elevated Nox4 expression, although Nox2 was essential for the development of contractile dysfunction and fibrosis (Byrne et al. 2003; Grieve et al. 2006). Interestingly, further studies found that mice with cardiomyocyte-targeted Nox4 overexpression, which exhibit elevated levels of H_2O_2 , showed reduced LV hypertrophy, dilatation, and fibrosis with preserved contractile function after both pressure overload and MI, indicating that Nox4 NADPH oxidase may be cardioprotective, at least in this setting (Zhang et al. 2009; Zhang et al. 2010). However, a recent study reported that Nox4 NADPH oxidase-derived ROS promote cardiac remodeling associated with type 1 diabetes (Maalouf et al. 2012). The role of NADPH oxidases in cardiac remodeling is therefore complex, with differences in regulation, activation, subcellular localization, and ROS generation appearing to dictate distinct isoform-specific actions (Dikalov et al. 2008; Brown and Griendling 2009).

Mitochondria

Mitochondria represent not only an important enzymatic source of ROS but also a major target for ROS-mediated damage in the myocardium. Under normal physiological conditions, low levels of $O_2^{\cdot-}$ are produced as a by-product of mitochondrial respiration via single electron transport from FADH and NADH to molecular oxygen, catalyzed by carriers located on the inner mitochondrial

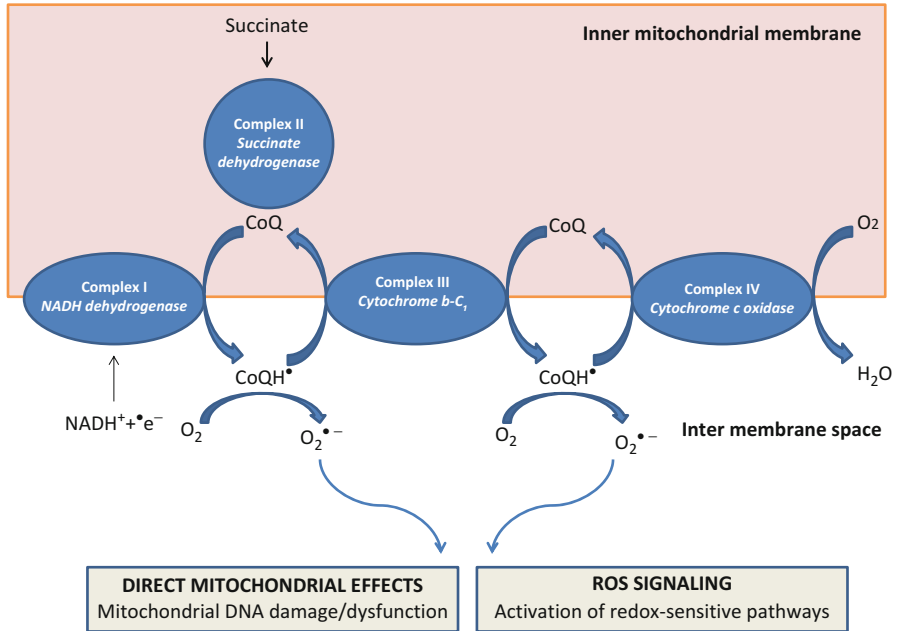


Fig. 40.3 Generation of $O_2^{\bullet-}$ by mitochondria. The mitochondrial electron transport chain is a sequence of carrier complexes, I, II, III, and IV, which transport electrons gradually to O_2 . Coenzyme Q (CoQ) shuttles the electrons between complex I or II and III and further between III and IV. CoQ is a major source of $O_2^{\bullet-}$ in pathologic states because some of the electrons, which are transported from NADH or other compounds to O_2 , “escape” when $CoQH^{\bullet}$ reacts with O_2 to form $O_2^{\bullet-}$. This $O_2^{\bullet-}$ can then either exert direct detrimental effects on the mitochondria itself or mediate more widespread actions via activation of redox-sensitive signaling pathways. *FMN* flavin mononucleotide, *FAD* flavin adenine dinucleotide [Figure adapted from Sorescu and Griendling (2002)]

membrane (as shown in Fig. 40.3). These small amounts of ROS are then easily scavenged by endogenous antioxidant systems, primarily Mn-SOD (Sawyer and Colucci 2000). Due to their increased energy requirement cardiomyocytes have a particularly high density of mitochondria, and uncoupling of the mitochondrial respiratory chain in pathological conditions can consequently result in marked elevation in ROS production (Sorescu and Griendling 2002). Indeed, increased production of mitochondrial-derived ROS has been reported in several experimental models of cardiac remodeling and CHF. For example, in canine hearts subjected to chronic rapid ventricular pacing, ROS generation by mitochondrial complex I was shown to be markedly increased, resulting primarily in the formation of $\bullet OH$ (Ide et al. 1999; 2000). In a murine model of chronic MI, increases in ROS production were associated with mitochondrial DNA damage and defective electron transport, together with parallel increases in mitochondrial levels of 8-oxo-dGTPase, which is protective against ROS-mediated DNA damage

and may be a marker of mitochondrial oxidative stress (Ide et al. 2001; Tsutsui et al. 2001). Furthermore, gene-modified mice with mitochondrial-targeted overexpression of catalase or cardiac-specific overexpression of the mitochondrial antioxidant, thioredoxin-1, demonstrated reduced cardiac remodeling in response to chronic pressure overload, while mitochondrial peroxiredoxin-3 overexpression protected against post-MI ventricular remodeling (Yamamoto et al. 2003; Matsushima et al. 2006; Dai et al. 2012). Data relating to mitochondrial ROS production in clinical CHF are scarce, although one study reported markedly increased expression of Mn-SOD in failing hearts of patients with nonischemic dilated cardiomyopathy, in parallel with elevated production of $O_2^{\bullet-}$ (Sam et al. 2005).

Dysfunctional NOS

In addition to its well-established role in regulation of vascular function, nitric oxide (NO) is also known to limit the development of experimental and clinical CHF by exerting specific effects on cardiac remodeling (GISSI-3. 1994; Ritchie et al. 1998; Kim et al. 1999). Indeed, ventricular remodeling in response to both chronic pressure overload and MI was exacerbated in gene-modified mice lacking global expression of endothelial NO synthase (NOS3) (Scherrer-Crosbie et al. 2001; Ruetten et al. 2005). Conversely, cardiomyocyte-specific NOS3 overexpression resulted in attenuation of pressure-overload-induced cardiac remodeling (Buys et al. 2007). However, under pathological conditions NOS3 may become uncoupled due to oxidation of its essential cofactor tetrahydrobiopterin (BH_4) by ROS (Landmesser et al. 2003). Uncoupling of NOS in CHF leads not only to a significant reduction in NO bioavailability but also to further increases in ROS production. Under physiological conditions, NOS3 catalyzes the conversion of L-arginine and oxygen to NO and citrulline. However, increased ROS production or deficiency of BH_4 or L-arginine may cause NOS3 to become uncoupled and produce $O_2^{\bullet-}$ rather than NO (Stuehr et al. 2001; Landmesser et al. 2003). Furthermore, uncoupled NOS3 has also been reported to potentiate mitochondrial ROS production by increasing mitochondrial nitration (Sud et al. 2008). In addition, NO itself, levels of which may be increased in CHF, can react with ROS leading to production of the powerful oxidant, peroxynitrite, and nitrosative stress (Stuehr et al. 2001; Landmesser et al. 2003; Nava et al. 1995). Indeed, decreased BH_4 levels in experimental hypertension and pressure overload have been shown to lead to NOS3 uncoupling and consequent increases in myocardial ROS production, cardiac remodeling, and contractile dysfunction (Takimoto et al. 2005; Silberman et al. 2010). The development of these changes was attenuated by co-treatment with BH_4 , which was also able to reverse preexisting cardiac hypertrophy and fibrosis (Takimoto et al. 2005; Moens et al. 2008; Silberman et al. 2010). However, recent data suggests that the clinical use of BH_4 in this setting may be limited by *in vivo* oxidation resulting in dose-dependent increases in competing levels of BH_2 (Moens et al. 2008; Crabtree et al. 2009).

Monoamine Oxidases

Monoamine oxidases (MAOs) are emerging as a significant source of ROS within the cardiovascular system. There are two isoforms, MAO-A and MAO-B, which are expressed on the outer mitochondrial membrane and catalyze the oxidative deamination of monoamines resulting in H₂O₂ production (Kaludercic et al. 2011). In the context of CHF, MAO-A is of particular interest as it primarily catabolizes epinephrine, serotonin, and norepinephrine, all of which are potent stimuli of cardiac remodeling. Indeed, recent studies have documented an important role for cardiac MAO-A-derived ROS both in vitro and in vivo. In rat neonatal and H9c2 cardiomyocytes, stimulated MAO-A-dependent ROS production was associated with cell apoptosis and hypertrophy, which were inhibited by the specific MAO-A inhibitor, clorgyline (Pchejetski et al. 2007; Kaludercic et al. 2010). In addition, myocardial MAO-A expression is increased in a Dahl salt-sensitive rat model of experimental CHF (Kong et al. 2005). Furthermore, chronic MAO-A inhibition has been found to protect against the development of pressure-overload-induced LV contractile dysfunction and dilatation, while acute ischemia-reperfusion injury was reduced in MAO-A-deficient mice (Pchejetski et al. 2007; Kaludercic et al. 2010). Although the majority of studies to date have focused on the MAO-A isoform, myocardial expression of MAO-B is also reported to be increased in dogs with chronic rapid ventricular pacing-induced CHF (Ojaimi et al. 2007), suggesting that ROS produced by this isoform may also play an important role in cardiac remodeling.

Myeloperoxidase

Myeloperoxidase is a major enzymatic source of ROS produced by activated leukocytes, including neutrophils and monocytes, which play a key role in myocardial inflammation and subsequent modulation of cardiac remodeling (Hampton et al. 1998; Heymans et al. 1999). Myeloperoxidase generates oxidants such as hypochlorous acid from H₂O₂, chloride ions, and monochloramines that are cytotoxic and are produced under physiological conditions as part of the respiratory burst of host defense which functions to destroy invading bacteria and pathogens (Klebanoff 2005). Similar to NADPH oxidases, this enzyme plays a key basal function, but levels are increased in the ischemic heart, where it has been shown to play an important role in ventricular dilatation and rupture post-MI via oxidation of plasminogen activator inhibitor-1 (PAI-1), an established mediator of ischemic cardiac remodeling (Heymans et al. 1999; Askari et al. 2003; Nahrendorf et al. 2008). Indeed, increased circulating myeloperoxidase levels have been suggested as both a predicative and diagnostic marker of acute heart failure (Sinning et al. 2008; Tang et al. 2011). Myeloperoxidase is also known to combine with amino acids to produce reactive aldehydes, such as acrolein, which may be important in the pathophysiology of cardiac remodeling (Srivastava et al. 2002; Ismahil et al. 2011). Interestingly, a recent study reported that myeloperoxidase-deficient mice

were specifically protected against chronic post-MI remodeling but not acute infarct remodeling, suggesting that myeloperoxidase-derived oxidants do not mediate tissue necrosis after MI but may be an important mediator of heart failure progression (Vasilyev et al. 2005).

Cross Talk Between ROS Sources

Interestingly, it is becoming increasingly apparent that cross talk between different cardiovascular ROS sources and the emerging concept of ROS-induced ROS release are likely to play a significant role in overall regulation of cardiac remodeling (Kimura et al. 2005; Dikalov 2011). Given the highly specific nature of ROS signaling, it is thought that such interactions may exert spatial and temporal control over regional ROS production and facilitate localized amplification of ROS-dependent cellular processes (Zinkevich and Gutterman 2011). For example, mitochondrial ROS have been shown to stimulate phosphatidylinositol 3-kinase (PI3K)-mediated Rac1 translocation resulting in activation of Nox1, whereas converse activation of mitochondrial $O_2^{\bullet-}$ production occurs in response to NADPH oxidase-derived ROS in a Ca^{2+} -dependent manner (Lee et al. 2006; Hawkins et al. 2007). Indeed, cross talk between mitochondria and NADPH oxidase-derived ROS is thought to play a key role in Ang II-mediated vascular redox responses (Wosniak et al. 2009). Furthermore, NADPH oxidases are known to modulate the activity of xanthine oxidase by regulating its conversion from xanthine dehydrogenase, and to oxidize BH_4 which results in uncoupling of NOS3 and conversion of this NO-producing enzyme to one that generates primarily $O_2^{\bullet-}$ (Landmesser et al. 2003; McNally et al. 2003). Such cross talk between different ROS sources adds further complexity to ROS signaling and is likely to underlie many of their highly specific actions in the setting of cardiac remodeling and CHF.

Redox-Sensitive Pathways in Remodeling and Failure

It is clear that a number of ROS sources, produced in response to multiple stimuli, are important in regulating important aspects of cardiac remodeling and CHF (as summarized in Fig. 40.4). Indeed, several key signaling pathways involved in disease pathogenesis have been identified as being redox sensitive. Although the precise underlying mechanisms remain to be established, it is clear that ROS are capable of exerting highly focused actions on the remodeling phenotype via specific modulation of defined signaling pathways, and these will now be discussed in detail.

Serine/Threonine Protein Kinases

Protein kinases are major regulators of signal transduction that catalyze phosphorylation of other proteins, thereby controlling their activity. Primary targets of

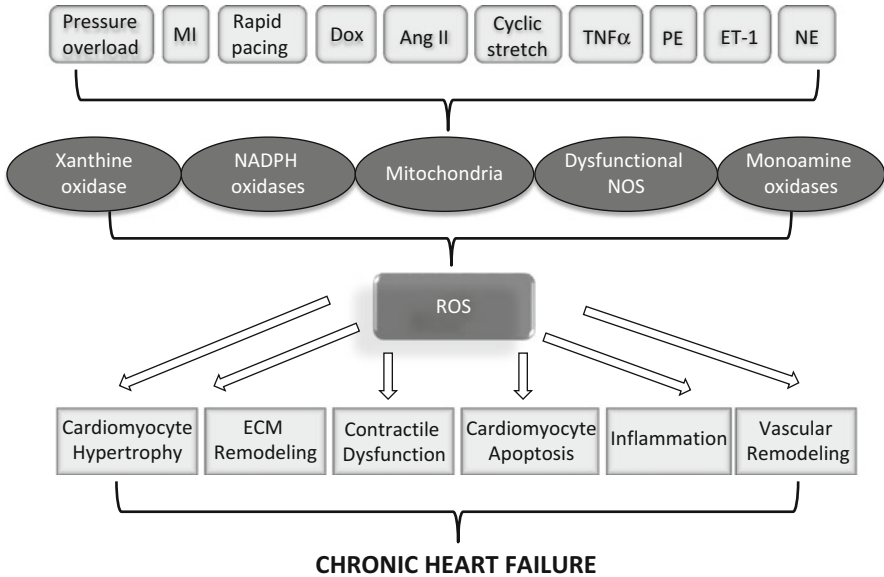


Fig. 40.4 Summary of the actions of major cardiac ROS sources in CHF. Several major cardiac ROS sources may be produced in response to multiple stimuli, which then exert important downstream actions on specific components of the cardiac remodeling phenotype, ultimately leading to CHF development. *Ang II* angiotensin II, *Dox* doxorubicin, *ECM* extracellular matrix, *ET-1* endothelin-1, *MI* myocardial infarction, *NE* norepinephrine, *PE* phenylephrine; *TNF α* tumor necrosis factor- α .

protein kinases are transcription factors which modulate intracellular signaling via specific alteration of downstream gene expression/activity (Force et al. 1999). The most important protein kinases in the heart are the serine/threonine subfamily, which act by promoting phosphorylation of the OH group of serine or threonine residues on target proteins (Cheng and Force 2010). Of these, mitogen-activated protein kinases (MAPKs) and protein kinase B (Akt) play a well-established role in cardiac remodeling and CHF and are known to be particularly sensitive to redox modulation (Feuerstein and Young. 2000; Sugden and Clerk 2006).

Mitogen-Activated Protein Kinases (MAPKs)

Cardiac MAPK Signaling

Mitogen-activated protein kinases (MAPKs) represent a large family of proteins that play important roles in signal transduction within the heart, where they are involved in regulation of a number of biological processes such as cell motility,

survival, apoptosis, proliferation, and differentiation. In cardiomyocytes, MAPK signaling is promoted by a number of stimuli, including G-protein-coupled receptor (GPCR) activation, receptor tyrosine kinases, oxidative stress, and growth factors, and contains a number of sequentially acting kinases which ultimately result in phosphorylation and activation of terminal effector kinases which transduce specific cellular actions (Robinson and Cobb 1997; Bueno et al. 2000). Several MAPK family subgroups have been identified, of which the major mammalian types appear to be extracellular signal-regulated kinases (ERK1/2), c-Jun NH₂-terminal kinases (JNK1, 2 and 3), and p38MAPK (α , β , δ and γ), all of which play key roles during both cardiac development and in disease (Robinson and Cobb 1997; Zhang et al. 2007). Indeed, activity of ERK1/2, JNK, and p38MAPK has been reported to be increased in both experimental and clinical CHF (Cook et al. 1999). However, the involvement of MAPKs in this setting appears to be particularly complicated as different components within each pathway may exert positive or negative effects during disease progression and may be damaging in one situation while beneficial in another (Rose et al. 2010). Nonetheless, it is clear that MAPK activation plays a key role in the pathogenesis of CHF and is critically involved in regulation of specific aspects of cardiac remodeling (Sano et al. 2001).

ROS Modulation of MAPK Signaling in Cardiac Remodeling

MAPKs are the most investigated family of signal transducer proteins in relation to ROS-dependent modulation of cardiac remodeling, with the three major mammalian MAPK subgroups being implicated in controlling important aspects of the underlying pathophysiological processes. However, the precise role that ROS modulation of MAPK signaling plays in overall regulation of cardiac remodeling remains controversial, with the current evidence highlighting species-, stimulus-, and model-specific effects of ROS-dependent activation of the major MAPK pathways. It is well established that several MAPKs are activated in a ROS-dependent manner in the heart. For example, cardiac p38MAPK signaling has been reported to be activated by both xanthine oxidase-derived ROS and by mitochondrial-derived ROS generated under hypoxic conditions (Kulisz et al. 2002; Gaitanaki et al. 2006). Increased NADPH oxidase expression/activity and O₂^{•-} production has also been shown to be associated with activation of ERK1/2 and p38MAPK and JNK1/2 in an experimental guinea-pig model of chronic pressure overload (Li et al. 2002). To date, the majority of experimental studies in relation to ROS-dependent modulation of MAPK signaling in cardiac remodeling and CHF have been performed *in vitro*. In isolated rat cardiomyocytes, both ET-1- and PE-induced hypertrophy were associated with increased ERK activity, which were both attenuated by acute antioxidant treatment, suggesting a key role for ROS-dependent modulation of ERK signaling in this setting (Amin et al. 2001; Tanaka et al. 2001). Further studies in these cells have suggested that NADPH oxidase-derived ROS may specifically activate the ERK1/2 signaling pathway (Xiao et al. 2002). Similarly, hypertrophy of neonatal rat cardiomyocytes

induced by Rac1, which is known to be critical for Nox2 NADPH oxidase activity, was found to involve activation of both ERK1/2- and JNK-dependent signaling pathways (Clerk et al. 2001). Additional studies employing the same cell model have demonstrated that both H₂O₂- and aldosterone-induced apoptosis and GPCR agonist-induced hypertrophy are associated with ROS-dependent activation of Apoptosis signal-regulating kinase-1 (ASK-1), an upstream regulator of MAPK signaling (Hirota et al. 2002; Hayashi et al. 2008; Zhang et al. 2011a). Interestingly, it appears that H₂O₂ may exert differential concentration-dependent actions on cardiomyocyte remodeling. Low concentrations of H₂O₂ (10 μM) were found to induce hypertrophy of primary rat ventricular cardiomyocytes, with associated activation of ERK1/2 but not JNK, p38MAPK, or Akt, whereas increased concentrations (100–200 μM) produced an apoptotic response which was related to additional activation of JNK, p38MAPK, and Akt with a further activation of ERK1/2 (Kwon et al. 2003). Complementary studies performed in H9c2 cardiomyocytes indicated that ROS-induced apoptosis was associated with activation of JNK, but not ERK or p38MAPK pathways, and that specific inhibition of JNK signaling resulted in significant attenuation of ROS-mediated cell death (Turner et al. 1998). In the same cell model, Ang II-induced cardiomyocyte apoptosis was shown to involve NADPH oxidase-dependent activation of p38MAPK (Qin et al. 2006). Studies such as these serve to highlight the co of ROS signaling and suggest that differential MAPK activation may play an important role in regulation of cardiomyocyte remodeling. Interestingly, it appears that ROS may also activate MAPK signaling in a paracrine manner, as Ang II-dependent ROS production in isolated rat cardiac fibroblasts was found to stimulate cardiomyocyte hypertrophy via IL-6-induced activation of p38 MAPK, ERK1/2, and JNK-1 (Sano et al. 2001). MMP-2, which plays a key role in regulating extracellular matrix turnover, was also shown to be activated in H9c2 cardiomyocytes exposed to doxorubicin via NADPH oxidase-mediated JNK, p38MAPK, and ERK1/2 signaling (Spallarossa et al. 2006). Furthermore, chronic isoproterenol infusion in rats induced myocardial ROS production and collagen accumulation, which was associated with activation of ASK-1, ERK1/2, JNK, and p38MAPK (Zhang et al. 2005), supporting the suggestion that ROS-dependent modulation of MAPK signaling may be important in regulation of extracellular matrix remodeling. In addition to exerting specific actions on cardiac remodeling, other studies indicate that ROS-dependent modulation of MAPK signaling may also be important in vascular remodeling, which is known to be a central factor in CHF progression. For example, NADPH oxidase-dependent ROS generation in response to ischemia/reperfusion has been shown to promote in vitro angiogenesis and angiogenic growth factor expression in porcine coronary artery endothelial cells via activation of ERK1/2 and Akt pathways (Chen et al. 2007). Interestingly, mitochondrial ROS generation in porcine aortic endothelial cells under hypoxic conditions was found to occur upstream of ERK2 activation and further NADPH oxidase-mediated ROS production (Schäfer et al. 2003). Furthermore, ROS-dependent activation of p38MAPK is thought to represent a key step in Ang II-induced hypertrophy of rat vascular smooth muscle cells (VSMC)

(Ushio-Fukai et al. 1998) and may also be important in the development of atherosclerosis, with additional involvement of NADPH oxidase-dependent JNK activation (Viedt et al. 2000).

Akt

Cardiac Akt Signaling

Akt (also known as protein kinase B, PKB) is a protein kinase belonging to the serine/threonine family, which is stimulated by various receptor tyrosine kinases in a PI3K-dependent manner. In the myocardium, Akt activation has been shown to be regulated by a variety of physiological factors (e.g., exercise training, insulin/nutritional status) and under pathological conditions, such as chronic pressure overload, where it plays an important role in regulation of cardiomyocyte growth and hypertrophy (Walsh 2006). However, the precise function that Akt signaling plays in regulation of cardiac remodeling remains controversial, with several lines of evidence suggesting a protective role, while others indicate that Akt activation may exert maladaptive actions in this setting. For example, Ang II-induced H9c2 cardiomyocyte hypertrophy has been shown to be associated with Akt activation which was abolished by pretreatment with a recombinant adenoviral vector targeting Akt (Hingtgen et al. 2006). Furthermore, in a mouse model of chronic pressure overload, cardiac hypertrophy and associated Akt activation was attenuated by intramyocardial injections of an Akt-targeting viral vector (Hingtgen et al. 2010). However, it has also been reported that chronic blockade of Akt signaling in rats exacerbates pathological hypertrophy in response to both MI and pressure overload but inhibits physiological hypertrophy induced by exercise training (Buss et al. 2012). In addition, intracoronary gene delivery of Akt1 appears to be protective against the development of experimental doxorubicin-induced cardiac dysfunction (Taniyama and Walsh 2002), suggesting that Akt activation may play an adaptive role.

ROS-Dependent Akt Activation in Cardiac Remodeling

Although the precise actions of Akt signaling in cardiac remodeling and CHF remain incompletely understood, it is clear that Akt activation in this setting is redox sensitive and subject to differential regulation by several ROS types and sources. Interestingly, ROS have been shown to both promote and inhibit Akt activation, which are likely to contribute to their highly specific actions on distinct components of the cardiac remodeling phenotype, further illustrating the complexity of ROS signaling in this situation. Several studies have shown ROS to mediate activation of Akt, thereby exerting maladaptive actions on key remodeling processes. For example, H₂O₂-induced H9c2 cardiomyocyte apoptosis was found to occur via activation of the PI3K-Akt pathway, by promotion of disulfide bond formation between the Cys-297 and Cys-311 residues of Akt and consequent dephosphorylation, effects which were inhibited by both antioxidant treatment and endogenous overexpression of glutaredoxin (Murata et al. 2003; Zhu et al. 2011).

Similarly, agonist-induced hypertrophy of both rat neonatal cardiomyocytes and H9c2 cells is associated with Akt activation and has been shown to be both dependent upon increased Nox2 NADPH oxidase activity and sensitive to overexpression of endogenous SOD (Hingtgen et al. 2006; Häuselmann et al. 2011). Ang II-induced endothelial dysfunction also appears to be dependent on activation of both Nox2 NADPH oxidase and Akt signaling (Thakur et al. 2010). Further evidence for specific activation of Akt signaling by ROS comes from a recent study reporting that development of pressure-overload cardiac hypertrophy in rats was dependent on both increased ROS generation and phosphorylation of glycogen synthase kinase-3 β (GSK-3 β), a known substrate of Akt, with established pro-hypertrophic actions (Planavila et al. 2008).

ROS-Dependent Inactivation of Akt in Cardiac Remodeling

Although the above studies clearly indicate that ROS may exert their established maladaptive remodeling actions via activation of Akt signaling, other contrasting reports suggest that these effects may occur secondary to negative regulation of Akt by ROS. Interestingly, doxorubicin-induced cardiotoxicity was exacerbated in transgenic mice overexpressing Nrdp1 via enhanced ROS-mediated inhibition of Akt signaling (Zhang et al. 2011b), while cardiac hypertrophy in a rat model of experimental hyperthyroidism was associated with increased H₂O₂ activity generation and downregulation of the Akt pathway (Fernandes et al. 2011). Similarly, elevated ROS production in rats subjected to chronic MI was accompanied by decreased phosphorylation of both Akt and GSK-3 β in association with significant cardiac hypertrophy and contractile dysfunction (Schenkel et al. 2010). Furthermore, norepinephrine-induced apoptosis of rat neonatal cardiomyocytes was shown to involve ROS-mediated inhibition of Akt signaling (Fu et al. 2006), suggesting that Akt may confer cardioprotective actions. Indeed, in the setting of acute ischemia/reperfusion injury, increased ROS generation is regarded as being an important trigger for several cardioprotective mechanisms, which in this case involves activation, rather than inhibition of Akt (Wang et al. 2011).

Transcription Factors

Transcription factors are proteins which bind to specific DNA sequences on target genes, thereby mediating important downstream effects of many intracellular signaling pathways (Latchman. 1997). Several transcription factors are known to be activated during cardiac remodeling and CHF, including nuclear factor- κ B (NF- κ B), activator protein-1 (AP-1), nuclear factor of activated T cells (NFAT), myocyte enhancer factor-2 (MEF-2), cAMP response element-binding protein (CREB), GATA-4/6, and CCAAT-enhancer-binding protein β (C/EBP β), and to play important roles in this setting (Watson et al. 2010; Boström et al. 2010; Liu et al. 2012). Of these, NF- κ B and AP-1 appear to be particularly redox sensitive and to play a significant part in signal transduction during the development and progression of cardiac remodeling.

Nuclear Factor- κ B (NF- κ B)

Cardiac NF- κ B Signaling

The NF- κ B mammalian superfamily consists of five members separated into two groups based on divergence of the C terminus, known as the “Rel” subfamily (RelA, RelB, c-Rel) and the “NF- κ B” subfamily (p50, p52). Rel subfamily members possess C-terminal transcriptional activating domains (TADs) which recruit basal transcriptional machinery to target promoter/enhancer regions, whereas the NF- κ B subfamily members are deficient in TAD and thus require dimerization of Rel subfamily members for transcriptional activation (Gordon et al. 2011). Activation of NF- κ B triggers its release from the inhibitory protein, I κ B, which facilitates translocation to the nucleus. This can occur through various signaling mechanisms that have been subdivided into canonical and noncanonical pathways, with the former usually stimulated by TNF- α activating the most common NF- κ B dimer between p65 and p50, while the latter activates a RelB/p52 dimer. Within the heart, the canonical pathway represents the most investigated aspect of NF- κ B signaling, with little information available concerning the role of the noncanonical pathways. However, there is some evidence for the existence of cross talk within NF- κ B signaling as ligands for the noncanonical pathway have been shown to activate the canonical pathway. Activation of NF- κ B signaling has become inexorably linked with the development and progression of cardiac remodeling and CHF in a number of settings and is considered to be the major driver of myocardial inflammatory signaling via activation of downstream mediators such as TNF- α , iNOS, COX-2, IL-1 β , and IL-6 (Gordon et al. 2011; Hamid et al. 2011). In the clinical setting, increased NF- κ B activation has been demonstrated in peripheral blood leukocytes from CHF patients, while a genetic polymorphism which promotes NF- κ B activity and is associated with a more severe CHF phenotype has also been identified (Frantz et al. 2004; Jankowska et al. 2005; Santos et al. 2010). Activation of NF- κ B signaling has also been reported in commonly employed murine experimental models of CHF, such as coronary artery ligation, pressure overload, and streptozotocin diabetes (Hamid et al. 2009; Zelarayan et al. 2009; Rajesh et al. 2010). Gene-modified mice deficient in the c-Rel NF- κ B subunit were protected against Ang II-induced cardiac hypertrophy and fibrosis, while mice with cardiac-specific myotrophin overexpression exhibited myocardial hypertrophy which was markedly attenuated by *in vivo* silencing of NF- κ B (Gupta et al. 2008; Gaspar-Pereira et al. 2012). Furthermore, targeted disruption of the p50 subunit of NF- κ B in mice subjected to experimental MI was found to reduce reactive cardiac hypertrophy, fibrosis, chamber dilatation, and contractile dysfunction (Frantz et al. 2006; Kawano et al. 2006). Similarly, in the same model, chronic treatment with IMD-0354, a phosphorylation inhibitor of I κ B, attenuated post-MI geometric remodeling and associated inflammation (Onai et al. 2007). Indeed, NF- κ B is known to mediate pro-inflammatory signaling in cardiac remodeling associated with both diabetes and MI, where it is activated by TNF- α and IL-1 (Rajesh et al. 2010; Van der Heiden et al. 2010). Although the above studies and numerous others suggest that

activation of NF- κ B promotes pathological cardiac remodeling, a recent report indicates that NF- κ B signaling may also be protective. Interestingly, mice with cardiac-specific deletion of IKK β , a subunit of the IKK complex which is essential for NF- κ B activation, demonstrated significantly worse contractile dysfunction, hypertrophy, fibrosis, and apoptosis in response to chronic pressure overload compared to controls (Hikoso et al. 2009), suggesting that IKK β /NF- κ B signaling may perform a protective function, at least in this setting.

ROS Modulation of NF- κ B Signaling in Cardiac Remodeling

Over 20 years ago, H₂O₂ was shown to activate NF- κ B in human T cells at micromolar concentrations (Schreck et al. 1991). Since this initial report of redox modulation of NF- κ B signaling, it has become apparent that NF- κ B may be activated by various ROS types from different sources and that this plays a key role during the development and progression of cardiac remodeling. For example, in neonatal rat cardiomyocytes, glycated proteins, which are associated with diabetic cardiomyopathy, were found to increase NF- κ B activity via activation of Nox2 NADPH oxidase-derived O₂^{•-} (Zhang et al. 2006). In isolated perfused rat hearts, xanthine oxidase-derived ROS induced contractile dysfunction which was associated with NF- κ B activation (Turan et al. 2005). Furthermore, in obese db/db diabetic mice, increased mitochondrial free radical production and parallel LV dilatation and contractile dysfunction were attenuated by the NF- κ B inhibitor pyrrolidine dithiocarbamate (PDTC) (Rajesh et al. 2010). NF- κ B also appears to exert important ROS-dependent actions on specific components of the cardiac remodeling phenotype. Hypertrophy of rat neonatal cardiomyocytes in response to both GPCR agonists and TNF- α is reported to be mediated via NF- κ B activation, while sustained NF- κ B activity was shown to cause apoptosis of H9c2 cardiomyocytes (Higuchi et al. 2002; Hirotani et al. 2002; Hamid et al. 2009). Furthermore, Ang II-induced cardiac fibrosis in mice is associated with Nox2 NADPH oxidase-dependent NF- κ B activation (Johar et al. 2006). Similarly, aldosterone-induced cardiac fibrosis and inflammation were attenuated by chronic treatment with PDTC, a specific inhibitor of NF- κ B (Sun et al. 2002). Interestingly, exogenous and endogenous antioxidants appear to be effective at targeting NF- κ B signaling and reducing experimental cardiac remodeling. For example, GPCR-induced cardiomyocyte hypertrophy in rat neonatal cells was significantly reduced by a number of antioxidants, including N-acetylcysteine and vitamin E, which was associated with parallel decreases in NF- κ B activity (Hirotani et al. 2002). Intramyocardial gene transfer of Cu/Zn SOD in a murine model of pressure overload has also been demonstrated to ameliorate NF- κ B activation and cardiac hypertrophy (Hingtgen et al. 2010). Similarly, augmented pressure-overload-induced contractile dysfunction and remodeling observed in mice with cardiac-specific deletion of IKK β was thought to occur secondary to reduced expression/activity of manganese SOD (Hikoso et al. 2009). Furthermore, PDTC, which is not only a pharmacological inhibitor of NF- κ B but also a potent antioxidant, attenuated the development of cardiac hypertrophy in rats subjected to chronic pressure overload and those with spontaneous hypertension (Li et al. 2004; Gupta et al. 2005).

Activator Protein-1 (AP-1)

Cardiac AP-1 Signaling

AP-1 is a heterodimer with the major components, c-Jun and c-Fos, which is activated by multiple stimuli (e.g., cytokines, growth factors, stress), and is involved in a number of physiological processes including cell proliferation, differentiation, and apoptosis. The wide-ranging actions of AP-1 may be explained by its complex structure, consisting of dimeric basic region-leucine zipper (bZIP) proteins belonging to Jun, Fos, ATF, and JDP subfamilies which recognize TPA response elements of several target genes (Shaulian and Karin 2002; Hess et al. 2004). Activation of AP-1 has been demonstrated in experimental models of MI, pressure overload, and isoproterenol-induced cardiac hypertrophy and in human CHF patients with ischemic or dilated cardiomyopathy (Takemoto et al. 1999; Frantz et al. 2003; Chen et al. 2009; Garlie et al. 2011). In addition, *Ephx2* was recently identified as a susceptibility gene for experimental CHF in spontaneously hypertensive rats, which is thought to act by increasing AP-1 binding and activation (Monti et al. 2008). Furthermore, mutation of the consensus binding site for AP-1 on the AT1a receptor, which is a major mediator of cardiac Ang II signaling, was shown to abolish pressure-overload-induced hypertrophy in rats (Herzig et al. 1997). Importantly, AP-1 activation is associated with several aspects of the cardiac remodeling process. In rat neonatal cardiomyocytes, hypertrophy induced by the GPCR agonists, ET-1 and PE, was linked to c-Jun-dependent activation of AP-1 (Irukayama-Tomobe et al. 2004; Hilfiker-Kleiner et al. 2006). Indeed, the ET-1 gene itself has binding sites for AP-1 within its promoter region, suggesting that AP-1 may also contribute to ET-1 induction (Sakai et al. 2002). Isolated rat hearts subjected to acute ischemia/reperfusion also demonstrated a progressive increase in AP-1 activity which was associated with apoptotic cell death (Maulik et al. 2000). Furthermore, cardiac fibrosis both induced by streptozotocin treatment and in transgenic rats overexpressing the human renin and angiotensinogen genes was associated with AP-1 activation (Fiebeler et al. 2001; Aragno et al. 2008). Pro-inflammatory IL-18, which mediates MMP induction and extracellular matrix degradation, also activates AP-1 in adult rat cardiomyocytes (Reddy et al. 2010).

ROS Modulation of AP-1 Signaling in Cardiac Remodeling

It is well established that AP-1 activation is redox sensitive, with ROS, in particular $O_2^{\cdot-}$ and H_2O_2 , reported to induce expression of the AP-1 components, c-Fos and c-Jun (Peng et al. 1995; Cheng et al. 1999; Yang et al. 2005). AP-1 may also be activated secondary to upstream redox-sensitive modulation of the major MAPKs, JNK, ERK, and p38MAPK (Karin 1995; Taimor et al. 2001; Shaulian and Karin 2002), which themselves play an important role in ROS signaling in cardiac remodeling and CHF (see above). It is clear that ROS specifically derived from several important cardiac sources are capable of activating AP-1. In rat cardiomyocytes, the pro-hypertrophic GPCR agonist Ang II was found to increase AP-1 activity via activation of Nox2 NADPH oxidase, which occurred upstream of $TGF\beta_1$ induction and was dependent on p38MAPK (Wenzel et al. 2001; Wu et al. 2005).

Mitochondrial-derived $O_2^{\cdot-}$ also regulates TNF- α -induced activation of both NF- κ B and AP-1 in human myelogenous leukemia cells (Higuchi et al. 2002), while xanthine oxidase-induced rat cardiac fibroblast proliferation and associated induction of MMP gene expression is mediated by increased AP-1 activity (Xie et al. 2009). ROS-dependent activation of AP-1 also appears to play a key role in modulating specific aspects of cardiac remodeling. For example, Ang II-induced hypertrophic growth of rat neonatal cardiomyocytes was found to be dependent on ROS-mediated induction of AP-1 activity and expression of c-Fos and c-Jun (Li et al. 2005). Interestingly, norepinephrine used at pro-hypertrophic and proapoptotic concentrations in H9c2 cardiomyocytes caused differential induction of the AP-1 components, FosB and Fra-1, which was ROS dependent (Gupta et al. 2006), indicating that distinct redox regulation of AP-1 signaling may play an important role in cardiac remodeling, at least in this setting. Ang II-induced proliferation of neonatal rat cardiac fibroblasts is also dependent on ROS, which mediate AP-1 binding to the promoter region of ET-1 (Cheng et al. 2003). An important function for ROS-induced AP-1 activation in cardiac fibrosis is supported by data from a mouse model of cardiac ischemia-reperfusion injury indicating that MMP-2 induction in this situation occurs through the AP-1 components FosB and JunB and is dependent on hydroxyl radical generation (Alfonso-Jaume et al. 2006). Furthermore, cardiac fibrosis and ROS production in STZ-treated rats was associated with parallel AP-1 activation which was attenuated by dehydroepiandrosterone (DHEA), which is known to prevent hyperglycemia-induced oxidative damage (Aragno et al. 2008). Acute ischemia-reperfusion in dogs also leads to ROS-dependent endothelial induction of pro-inflammatory monocyte chemotactic protein-1 (MCP-1) via activation of AP-1 and NF- κ B (Lakshminarayanan et al. 2001). The apparent importance of redox modulation of AP-1 activity in cardiac remodeling is confirmed by several studies demonstrating that associated activation of AP-1 may be attenuated by antioxidants. In experimental models of cardiac ischemia-reperfusion, both recombinant adenoviruses expressing Cu/ZnSOD or Mn-SOD and the oxygen radical scavengers *N*-(2-mercapto-propionyl)-glycine (MPG) and DMTU inhibited acute AP-1 activation with parallel reductions in infarct size and cardiomyocyte apoptosis (Maulik et al. 1999; Fan et al. 2002; Yang et al. 2003). In addition, norepinephrine-induced expression of the AP-1 components FosB and Fra-1 in H9c2 cardiomyocytes was suppressed by both the H_2O_2 scavenger, catalase, and the SOD mimetic, MnTMPyP (Jindal and Goswami 2011). Interestingly, however, basal expression of FosB and Fra-1 was increased by MnTMPyP but inhibited by catalase, indicating that the precise nature of AP-1 regulation in this setting may be dependent on the type of ROS produced.

Novel Redox-Sensitive Signaling Pathways

In addition to the established redox-sensitive serine/threonine protein kinase and transcription factor-mediated pathways discussed above, it is becoming apparent that several other ROS-dependent signaling pathways may also be involved in cardiac remodeling. For example, CD40 ligand, which is structurally related to

TNF- α and expressed in T cells and platelets, is known to bind CD40 on B cells, macrophages, endothelial cells, vascular smooth muscle cells, and cardiomyocytes and so may mediate processes such as inflammatory activation and extracellular matrix remodeling (Grewal and Flavell 1997; Seko et al. 1998). Indeed, CD40 ligand is increased in patients with both acute and chronic heart failure (Ueland et al. 2005). Intriguingly, CD40 ligand activation is known to induce ROS production (possibly via NADPH oxidases) and induction of several downstream pathways including PI3 kinase, p38, Akt, ERK1/2, and NF- κ B and so may function as a master regulator of redox signaling in the heart (Chakrabarti et al. 2005; Chen et al. 2008; Xia et al. 2010). Furthermore, ROS have been shown to mediate cardiac hypertrophy induced by ouabain and related glycosides via modulation of Na⁺/K⁺ ATPase activity and induction of Src-, Ras-, and p42/44 MAPK-dependent signaling (Xie et al. 1999; Tian et al. 2003; Xie and Xie 2005). Indeed, administration of a monoclonal antibody against the cardiotonic steroid, marinobufagenin, which may act via similar mechanisms, reverses cardiac fibrosis in rats with chronic renal failure via a ROS-dependent mechanism (Haller et al. 2012).

Summary and Clinical Perspective

Taken together, it is clear that ROS-mediated modulation of downstream signaling plays a key role in regulating important components of the cardiac remodeling phenotype (as summarized in Fig. 40.5). Although the precise nature of these ROS-dependent actions remains unclear, it is becoming increasingly apparent that the underlying signaling mechanisms are extremely complex and that a number of factors, such as stimulus, type of ROS produced, concentration, cellular localization, and local antioxidant balance, need to be considered. Redox modulation of intracellular signaling appears to be particularly complicated, as evidenced by reports of concentration-dependent induction of MAPKs and AP-1 components by ROS, and differential activation or inactivation of Akt signaling, which may exert either adaptive or maladaptive actions on cardiac remodeling. Paradoxically, however, the complex nature of ROS signaling in this setting may offer hope for the development of novel specifically targeted strategies for the more effective treatment of CHF. Numerous investigations have reported beneficial effects of antioxidant treatment on experimental cardiac remodeling and CHF in animal models (Dhalla et al. 1996; Byrne et al. 2003; Hamblin et al. 2007). Some clinical studies have shown antioxidants such as α -tocopherol and ascorbic acid to reduce oxidative stress (Ghatak et al. 1996; Rössig et al. 2001), while others have reported cardiovascular benefits including reduced rate of nonfatal MI in patients with coronary artery disease (Stephens et al. 1996) and decreased cardiovascular events and mortality in women (Lee et al. 2005; Cook et al. 2007). However, the findings of clinical antioxidant trials in terms of long-term effects on patient symptoms and clinical outcome have been generally disappointing (Yusuf et al. 2000; Heart Protection Study Collaborative Group 2002), with some studies even reporting an increased risk of CHF (Lonn et al. 2002; Marchioli et al. 2006). Given the apparent complexity of myocardial ROS signaling,

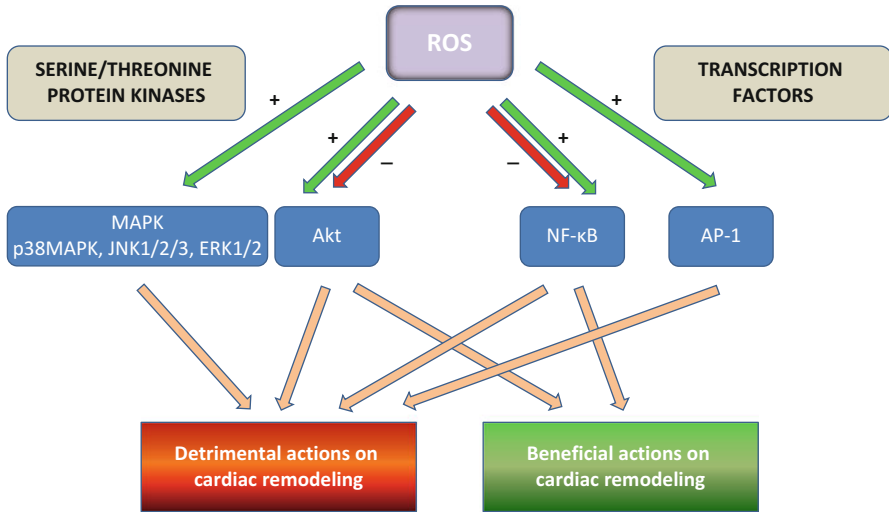


Fig. 40.5 Summary of the major redox-sensitive signaling pathways in cardiac remodeling. ROS modulation of downstream signaling plays a key role in mediating both adaptive and maladaptive cardiac remodeling. ROS-dependent signaling in this setting is extremely complex and is influenced by a number of factors. However, it appears that in general, mitogen-activated protein kinases (*MAPK*) and activator protein-1 (*AP-1*) are positively regulated by ROS and exert detrimental actions on cardiac remodeling, whereas Akt and nuclear factor- κ B (*NF- κ B*) may be both positively and negatively influenced by ROS and govern both adaptive and maladaptive aspects of cardiac remodeling. *ERK* extracellular signal-regulated kinase, *JNK* c-Jun NH₂-terminal kinase

it is perhaps not surprising that such nontargeted antioxidant approaches have had limited success. Not only do ROS play a critical physiological function as regulators of basal intracellular signaling, but they also alter the activity of numerous pathways to exert subtle modulatory effects on cardiac remodeling, which may be either adaptive or maladaptive. In this regard, it would clearly be attractive to selectively target detrimental aspects of the remodeling phenotype, such as fibrosis or cardiomyocyte apoptosis, while maintaining beneficial functions of ROS. Therefore, it is abundantly evident that a detailed understanding of the precise mechanisms by which ROS interact with key genes in order to specifically modulate downstream signaling in the heart is essential to facilitate the identification of novel therapeutic targets for more effective treatment of adverse cardiac remodeling and CHF.

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