PROSPECTS
Journal of Cellular Biochemistry 114:2437–2445 (2013)

Journal of Cellular Biochemistry

SWI/SNF in Cardiac Progenitor Cell Differentiation

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ABSTRACT

Cardiogenesis requires proper specification, proliferation, and differentiation of cardiac progenitor cells (CPCs). The differentiation of CPCs to specific cardiac cell types is likely guided by a comprehensive network comprised of cardiac transcription factors and epigenetic complexes. In this review, we describe how the ATP-dependent chromatin remodeling SWI/SNF complexes work synergistically with transcription and epigenetic factors to direct specific cardiac gene expression during CPC differentiation. Furthermore, we discuss how SWI/SNF may prime chromatin for cardiac gene expression at a genome-wide level. A detailed understanding of SWI/SNF-mediated CPC differentiation will provide important insight into the etiology of cardica defects and help design novel therapies for heart disease. J. Cell. Biochem. 114: 2437–2445, 2013.

KEY WORDS: SWI/SNF; CARDIAC PROGENITOR CELL; DIFFERENTIATION; ATP-DEPENDENT CHROMATIN REMODELNG

Heart disease remains the leading cause of death worldwide and presents a major economic burden to society. Congenital heart diseases (CHDs) are among the most common and most devastating birth defects in humans, affecting about 1% of live births (American Heart Association). Despite great progress in cardiac research, we have only limited knowledge on the etiology of CHDs and we still lack effective treatment for most of these patients. Meanwhile, myocardial infarction, commonly known as heart attack, results in permanent heart muscle damage or death, and is the number one killer of heart patients. Patients who survive a heart attack often develop heart failure symptoms and almost half of the affected patients die within 1 year from the onset of symptoms [Jessup and Brozena, 2003; Lopez et al., 2006]. Naturally, it is critical to understand the etiology of CHDs, and there is a high demand for developing novel therapeutic strategies to treat CHDs and heart failure.

The recent identification, characterization, and isolation of CPCs has provided new opportunities for understanding the etiology of CHDs and developing cell-based regenerative therapies for both CHDs and heart failure [Moretti et al., 2006; Wu et al., 2006; Bu et al., 2009]. The early CPCs are originated from lateral plate mesoderm soon after gastrulation. At E7.75 in mice, these CPCs formed cardiac crescent which is further classified into the first heart field (FHF) and the second heart field (SHF). CPCs from FHF and SHF then differentiated into cardiomyocyte and endocardial cells to form the linear heart tube at later stages. After a complex morphogenetic event and cell differentiation and maturation process, these CPCs formed the four chamber heart [Srivastava, 2006]. CPCs or CPC-like cells have also been identified in cardiac neural crest, epicardium, sinus venosus, as well as in adult heart [Beltrami et al., 2003; Cai et al., 2003; Oh et al., 2003; Kattman et al., 2006; Moretti et al., 2006; Wu et al., 2006; Yang et al., 2008; Blin et al., 2010].

The isolation and characterization of embryonic CPCs provides a new paradigm for studying CHDs. CHDs could be regarded as CPC diseases, that is, defects of CPCs in their specification, proliferation, migration, and differentiation likely lead to CHDs. In support of this notion, many transcription factors and signaling pathways that are essential for early cardiogenesis are also involved in CPC development and dysfunction of these factors and pathways is often associated with inherited CHDs [Keegan et al., 2005; Black, 2007; Bruneau, 2008; Kwon et al., 2009; Stevens et al., 2010]. Therefore, understanding the mechanisms of CPC development will shed new light on the etiology of CHDs. In particular, an ESC-based CPC specification, proliferation, and differentiation system combined with lineage tracing will be an attractive in vitro model system to study CHDs (Fig. 1) [Martin-Puig et al., 2008; Hansson et al., 2009].

CPCs are also ideal candidate cells for cell-based cardiac regenerative therapies to repair defective or damaged hearts through

Grant sponsor: US National Institutes of Health; Grant number: R01 HL109054-01; Grant sponsor: Stem Cell and Regenerative Medicine Consortium, HKU.

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Manuscript Received: 8 April 2013; Manuscript Accepted: 11 April 2013

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 19 April 2013

DOI 10.1002/jcb.24570 • © 2013 Wiley Periodicals, Inc.

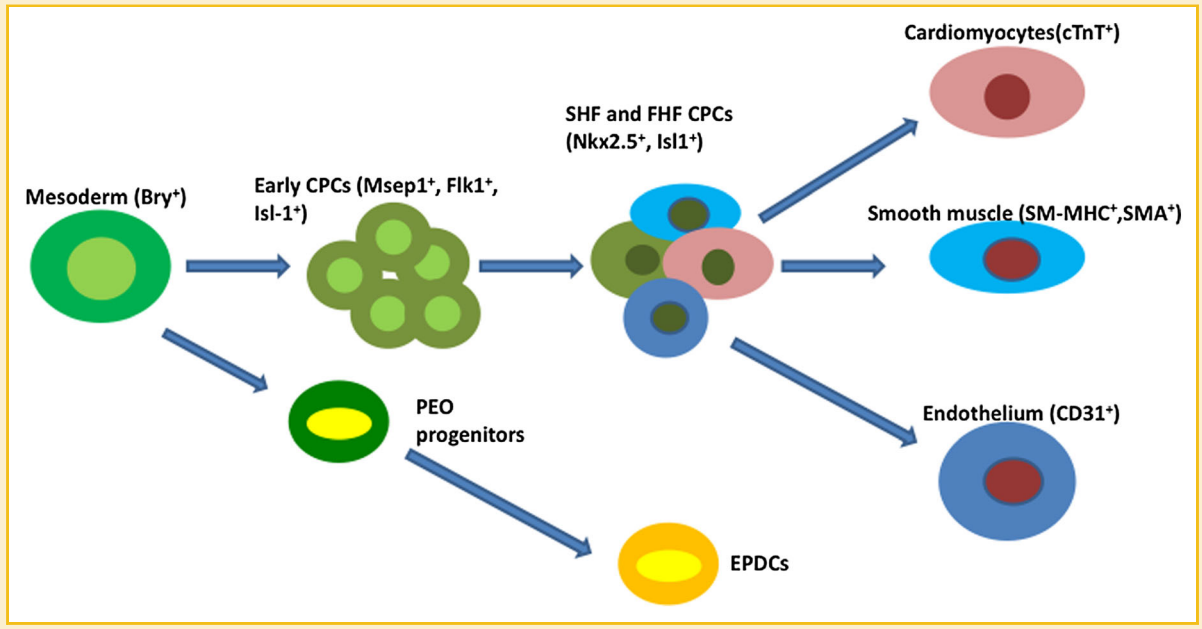

Fig. 1. A working model of heart cell lineage diversification from multipotent mesodermal progenitors. CPCs, cardiac progenitor cells; EPDCs, endothelial progenitor-derived cells; FHF, first heart field; PEO, proepicardial organ; SHF, second heart field. The molecular mechanisms guiding the proper differentiation of the cardiac lineages are poorly defined.

guided differentiation. CPCs derived from FHF and SHF are multipotent and capable of differentiating into cardiomyocytes, smooth muscle cells, and endothelial cells [Moretti et al., 2006; Wu et al., 2006; Bu et al., 2009], strongly suggesting that these cells are promising candidates in cell-based heart therapy. Due to their commitment to cardiac lineages, it is less likely that these cells will form teratomas or other unwanted cell types when injected into patient hearts. Numerous studies have shown that injecting CPCs into infarcted animal hearts improves the heart function [Blin et al., 2010; Christoforou et al., 2010]. However, it appears that the engrafted CPCs differentiated predominantly into early cardiomyocytes and the long-term effect needs to be further investigated. It is also conceivable that a more balanced differentiation of these CPCs into smooth muscle and endothelial cells to restore the coronary vasculature in the infarcted area may have a better therapeutic effect.

Therefore, to realize the full potential of CPCs in cardiac regenerative medicine, it is critical to understand the molecular mechanisms guiding directed differentiation of CPCs into desired mature cell types in the heart. Currently we have only limited knowledge about the CPC differentiation mechanisms which represents a roadblock in cell-based heart therapies for both CHDs and heart failure (Fig. 1).

MOLECULAR MECHANISMS GUIDING CPC DIFFERENTIATION

Though cardiogenesis has been extensively studied, the isolation and characterization of embryonic CPCs have only been accomplished in recent years and how these CPCs are programmed to differentiate into different cardiac lineages is largely unknown. It is very likely that the same transcription networks identified in cardiogenesis will function similarly in CPC proliferation, migration, and differentiation [Olson, 2006; Srivastava, 2006; Wu et al., 2008]. Furthermore, it has become increasingly clear that epigenetic regulation is also essential for gene regulation during development and on set of diseases [Zaidi et al., 2011]. There are three major types of chromatin modifications that play a key role in epigenetic regulation: DNA methylation, histone modification, and ATP-dependent chromatin remodeling [Suzuki and Bird, 2008; Ho and Crabtree, 2010; Zhou et al., 2011]. These chromatin modifications are catalyzed by a large number of protein complexes. One key group of the ATP-dependent chromatin remodeling suprerfamily is the SWI/SNF subfamily (Fig. 2A) [de la Serna et al., 2006; Wu et al., 2009]. To date, we have very limited knowledge about epigenetic regulation during cardiogenesis and CPC differentiation.

A comparison of epigenetic modifications between ESCs and CPCs may provide useful insights into the epigenetic mechanisms of CPC differentiation. In ESCs, epigenetic modifiers and chromatin signatures play a key role in pluripotency and differentiation. Studies have revealed that two classes of genes in ESCs may determine pluripotency and differentiation: active and "poised" genes [Mikkelsen et al., 2007; Rada-Iglesias et al., 2011]. Active genes include those master genes essential for ESC pluripotency and self-renewal. Many poised genes encode developmentally important factors whose expression is temporally silenced in ESCs but is readily activated during ESC differentiation. Both classes are marked by the presence of chromatin regulators p300 and BRG1 (catalytic subunit of SWI/SNF), monomethylation of histone H3 at lysine 4 (H3K4me1), and low nucleosomal density. These two classes are also marked by the enrichment of histone H3 lysine 4 trimethylation (H3K4me3), but they can be distinguished by the acetylation of histone H3 at lysine 27 (H3K27ac) in active genes, and the absence of H3K27ac and

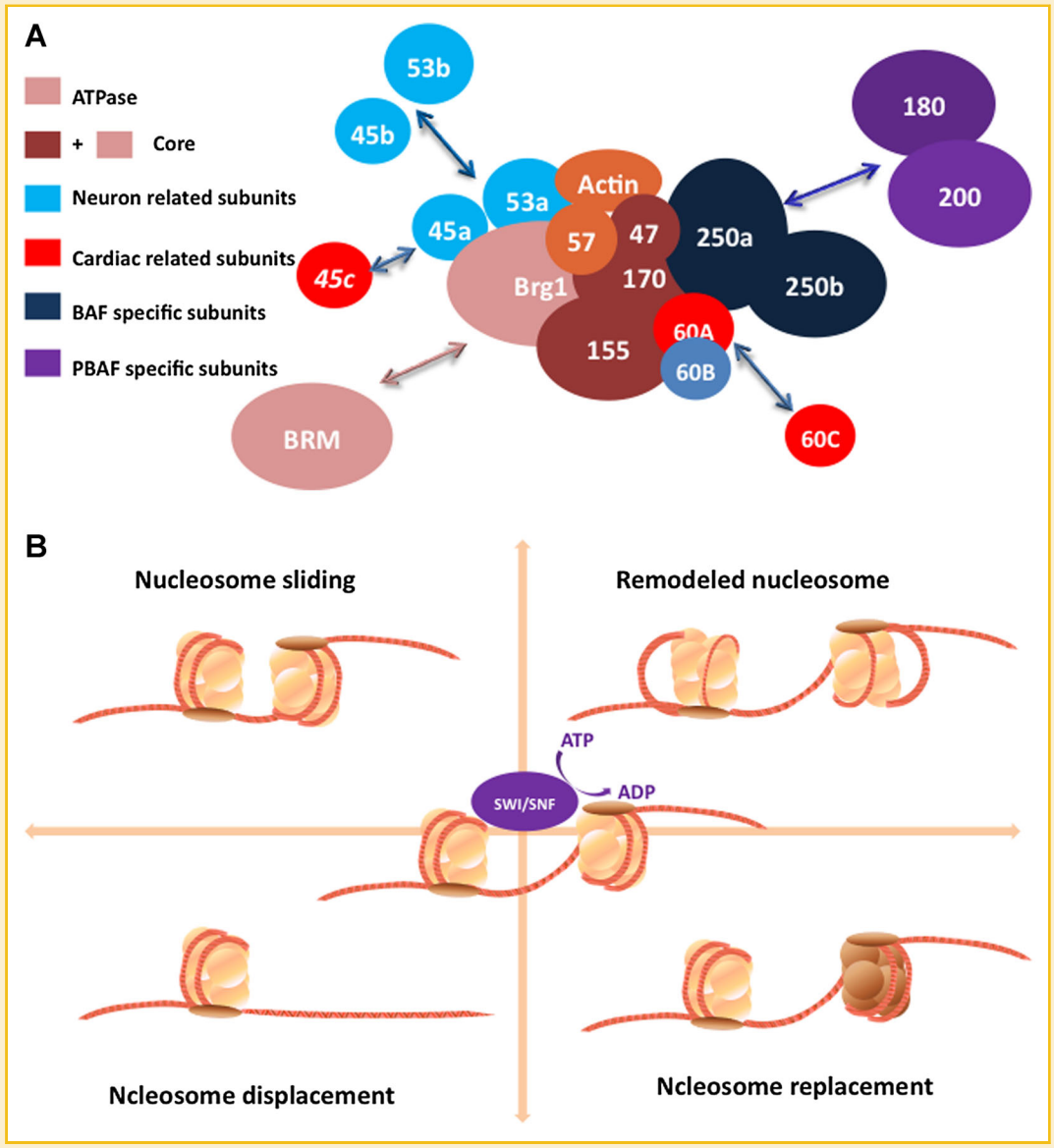

Fig. 2. Composition and enzymatic activities of SWI/SNF. A: Composition of SWI/SNF complex. The SWI/SNF are composed of 10–12 subunits. Certain subunits are interchangeable for tissue specific function. The assembles of each subunit in the figure do not represent their actual interaction. B: Working models of SWI/SNF-mediated chromatin remodeling. SWI/SNF use the energy of ATP hydrolysis to (1) move the nucleosomes along the DNA (upper left panel), (2) remodel nucleosomes and generate stable DNA loops within nucleosomes and dinucleosome-like species (upper right panel), (3) displace histones completely from the DNA (lower left panel), and (4) replace old histones with new ones (lower right panel).

enrichment of histone H3 lysine 27 trimethylation (H3K27me3) in poised genes [Mikkelsen et al., 2007; Creyghton et al., 2010; Rada-Iglesias et al., 2011].

It is likely that similar epigenetic factors and chromatin signatures are present in key genes in CPCs and are critical for the differentiation of CPCs into various lineages. Indeed, two studies have shown that during differentiation of embryonic stem cells to cardiovascular cells, distinct histone modifications are associated with key regulators of cardiogenesis, and this modification patterns can be applied to identify novel genes essential for cardiac development [Paige et al., 2012; Wamstad et al., 2012]. These studies strongly suggest that identifying the epigenetic factors and the chromatin signatures of key cardiac genes in CPCs could be an essential step towards elucidating the mechanisms of CPC differentiation. Remarkably, the chromatin signatures that define the two classes of genes described above are tightly associated with SWI/SNF (Fig. 2A), a major complex that plays a key role in various aspects of development, including heart development and disease.

SWI/SNF IN CARDIOGENESIS AND CPC DIFFERENTIATION

The SWI/SNF complexes are the prototype of ATP-dependent chromatin remodeling machines that are evolutionarily conserved

from yeast to human. The major enzymatic function of the SWI/SNF complexes is to remodel the nucleosome on DNA [Kingston and Tamkun, 2007] (Fig. 2B). The SWI/SNF complexes can function in several different ways: to move the nucleosomes along the DNA (Fig. 2B, upper left panel); to remodel nucleosomes and generate stable DNA loops within nucleosomes and dinucleosome-like species (Fig. 2B, upper right panel); to displace histones completely from the DNA (Fig. 2B, lower left panel); and to replace old histones with new ones by transferring the histone octamer to accepter DNA and exchanging H2A/H2B dimers between nucleosomes [Lorch et al., 1999; Whitehouse et al., 1999] (Fig. 2B, lower right panel). Even though these identified actions of SWISNF have been based mostly on in vitro experiments or in vivo analyses using small artificial DNA templates, these findings clearly suggest that SWI/SNF complexes play a significant role in regulating the chromatin plasticity required for CPC differentiation.

Mammalian SWI/SNF complexes are able to form various assemblies from different subunits [Cairns et al., 1996; Wang et al., 1996; Nie et al., 2000; Xue et al., 2000; Yan et al., 2005]. A series of studies show that SWI/SNF can be divided into two subfamilies. One includes yeast SWI/SNF, fly BAP, and mammalian BAF (SWI/SNF-A) complexes, the other includes yeast RSC, fly PBAP, and mammalian PBAF (SWI/SNF-B) complexes. BAF and PBAF share eight common subunits, and each has two unique ones. BAF250a (Arid1a)- and BAF250b (Arid1b) are uniquely associated with BAF, and BAF180 (Pb1) and BAF200 (Arid2) are uniquely associated with PBAF (Fig. 2A).

Recent studies also show that the stoichiometry and subunit composition of SWI/SNF are distinct in different cells and they change dynamically during differentiation. For examples, ES cells express a high level of BAF155, BAF250a, Brg1 but a low level of BAF170 and Brm [Yan et al., 2008; Ho et al., 2009]. These cell type specificity is also found in other developmental processes [Lickert et al., 2004; Lessard et al., 2007; Lange et al., 2008; Ho et al., 2009]. Moreover, the SWI/SNF subunits could be encoded by several genes to form a diversity of complexes which modulated different developmental and cell differentiation processes. ATPase Brg1 or Brm, with BAF170, BAF155, and BAF47 form the basis of the SWI/SNF complex. The other subunits including BAF45, BAF53, BAF60, BAF180, BAF200, BAF250, and actin, contain a set of variants to form the specific SWI/SNF complexes (Fig. 2A) [Wang et al., 1996; Wu et al., 2009].

Thus, it has been proposed that the SWI/SNF complex is uniquely deployed in each tissue to guide tissue-specific differentiation programs with its specific subunit composition and stoichiometry. Indeed, it appears that there is a cardiac specific SWI/SNF complex and its components have been shown to be essential for various stages of cardiogenesis. We discuss the functions of these subunits in cardiogenesis and CPC differentiation below.

BAF60C

BAF60c is a SWI/SNF component specifically expressed in heart and somites. RNAi knockdown of BAF60c caused abnormal cardiac and skeletal muscle development [Lickert et al., 2004]. The right ventricle marker Hand2 is absent and the outflow tract is shortened in BAF60c knockdown embryos suggesting that BAF60c plays a specific role in SHF expansion. Furthermore, BAF60c can promote the interaction of Brg1 with transcription factors such as Tbx5, Nkx2.5, and Gata4 in vitro. Intrigueingly, BAF60c, working together with Tbx5 and Gata4, could induce non-myogenic mesoderm into cardiomyocytes ex vivo [Takeuchi and Bruneau, 2009]. Moreover, Gata5 and BAF60c is able to promoter myocardial differentiation by directing cells migration to heart tube region [Lou et al., 2011]. Transplant assay in zebrafish showed that cardiac SWI/SNF can response to many key cardiogenic signals such as Wnt, Bmp as well as FGF signals to promote efficient cardiomyocyte differentiation suggesting SWI/SNF could have important roles in CPC formation. These results indicate that BAF60c contributes to the cardiac specificity of SWI/SNF and strongly suggest its potential key role in CPC differentiation.

BAF180

BAF180 is a unique polypeptide component of PBAF but not BAF. Our studies indicate that BAF180 is required for cardiac morphogenesis [Wang et al., 2004] and deletion of BAF180 in epicardial cells leads to impaired epithelial-to-mesenchymal- transition (EMT) of epicardium and causes failure of coronary vessel formation during embryonic development [Huang et al., 2008]. BAF180 deletion affects the α-SMA expression and coronary vessel formation, suggesting that BAF180 mediates the differentiation of endothelial progenitor-derived cells (EPDCs) into myofibroblast, endothelial cells, and smooth muscle cells. Although the mechanism of BAF180 in regulating CPC differentiation is not clear, it should be noted that BAF180/PBAF is required in retinoic acid (RA) signaling during heart development, presumably by serving as a cofactor for RXRα, PPARγ, and other RA-related nuclear receptors. Ablation of BAF180 in mouse embryos results in severe hypoplastic ventricle development and trophoblast placental defects, similar to those found in mice lacking RXRα and PPARγ. RA signaling in association with BAF180 have a critical role in controlling mesenchymal progenitor cell differentiation into smooth muscle and fibroblast lineages during cardiac development implying an important role of BAF180 in EPDCs.

BRG1

Mutation studies of Brg1 in different cardiac lineages showed complex functions of Brg1 during heart development [Stankunas et al., 2008; Hang et al., 2010]. Early deletion of Brg1 in myocardium leads to severe cardiac defects including thin myocardium and septum defect. These abnormalities are associated with the myocardial proliferation defect. Knockout of Brg1 causes down-regulated of Bmp10 and ectopic expression of p57kip2, which regulate proper myocardial proliferation. Rescue of Brg1 KO phenotypes by Bmp10 suggest that Bmp10 functions at downstream of Brg1 in myocardial proliferation. Moreover, Brg1 interacts with HDACs and PARPs to repress α-MHC and activate β-MHC during myocyte maturation during heart development. The Brg1/HDAC/PARP is shown to maintain β-MHC expression. Absence of Brg1 leads to premature differentiation of cardiomyocytes as indicated by expression of α-MHC. Inhibition of HDAC or PARP also causes premature differentiation of myocytes. These findings suggest that Brg1 control cardiac development by regulating different pathways in proliferation and differentiation.

Further studies have shown that a crosstalk between Brg1 and other epigenetic factors is essential for proper function of Brg1. The interaction of UTX and Brg1 promotes Brg1 binding to its cardiac target genes [Lee et al., 2012]. The association of UTX and Brg1 also significantly promotes the interaction of Brg1 with Tbx5 which is essential for cardiac specific function. Moreover, the recruitment of Brg1 to cardiac enhancers are reduced in UTX knockdown. Therefore, UTX recruits Brg1 to activate cardiac genes and proper interaction of SWI/SNF with various epigenetic factors are important for normal CPC differentiation.

BAF250A

The specific functions of SWI/SNF complex largely depend on its various components. BAF250a is a critical regulatory subunit in SWI/SNF. Our studies show that knockout of BAF250a in differentiaton cardiac lineages displayed multiple cardiac defects [Gao et al., 2008; Lei et al., 2012]. Single alelle deletion of BAF250a also leads to cardiac defects, indicating that BAF250a is also required in a dosage dependent manner. BAF250a is essential for proper differentiation of ES cells into beating cardiomyocytes during in vitro differentiaiton. Further analysis of the cardiac defects in BAF250a knockout embryos suggests that BAF250a regulates both differentation and proliferation of myocardium by mediating different pathways. Moreover, BAF250a is required for efficient Brg1 binding to several cardiac genes, such as MEF2c, NKX2.5, and BMP10. DNase hypersensitivity assays have shown that the function of SWI/SNF at cardaic targets is BAF250a dependent. Since BAF250a and BAF180 belongs to BAF and PBAF, it is likely that several cardiac specific SWI/SNF complexes may exist and play important roles during CPC differentiation.

PERSPECTIVE

SWI/SNF is required in various aspects of cariogenesis and plays a promising role in CPC differentiation. Future studies with a combined embryonic stem cell based in vitro differentiation systems and genetic, biochemical, and genome-wide sequencing technologies will enable us to fully understand SWI/SNF-mediated epigenetic mechanisms in guiding the proper differentiation of CPCs. We envision that future studies will systemally reveal the following: whether and how the dynamic regulation of SWI/SNF composition and stoichimetry mediates CPC development; what are the key transcription factors and epigentic factors that interact with SWI/SNF to change cell identity during CPC differentiation; more intriguely, how SWI/SNF primes chromatin structure at single gene, gene cluster, topological domains, as well as nuclear compartment to faciliate gene expression and CPC differentiation.

DYNAMICS OF SWI/SNF COMPLEX DURING CPC SPECIFICATION AND DIFFERENTIATION

One central question of SWI/SNF in CPC differentiation is how SWI/SNF itself is regulated. Numerous studies clearly indicate that tissue-specific SWI/SNFs are critical for the specification and differentiation of tissue-specific progenitors [Ho and Crabtree, 2010], including neuron and cardiac progenitors. The studies of BAF60c, BAF250a, Brg1, and BAF180 in heart development as discussed above have revealed a cardiac SWI/SNF (cSWI/SNF) or cSWI/SNFs in CPCs. The existence of cSWI/SNF is also supported by the findings that certain subunits are interchangeable and function in different cardiac cell stages. BAF60a is shown to interact with Tbx1 in embryos to maintain the self-renewal of CPCs [Chen et al., 2012]. Tbx1 regulates cardiac progenitor proliferation and inhibit differentiation and Tbx5 is required for cardiomyocyte differentiation. BAF60a/BAF complex exerts a specific function in maintain self-renewal of CPC via interact with Tbx1. On the other hand, BAF60c interacts with Tbx5 and their interaction is essential for the activation of cardiomyocyte structure genes, such as Tnnt2, Myh6, and Gja1 [Takeuchi and Bruneau, 2009]. Interestingly, BAF60a is expressed in the CPCs, and poorly expressed in cardiomyocytes, while BAF60c is highly expressed in cardiomyocytes and smooth muscle cells, indicating that BAF complexes go through a subunit change during CPC differentiation and interact with different transcription factors to acquire cardiac specificity. In addition, BAF45c has also been identified as a component of cSWI/SNF [Lange et al., 2008].

Looking forward, we envision that an integrated approach in genetics, biochemistry, and stem cell biology will yield a comprehensive picture of cSWI/SNF function in CPC differentiation. The development of ESC-based in vitro CPC systems combined with conventional biochemical purification techniques and state-of-the-art proteomic approach will greatly clarify the dynamic changes of cSWI/SNF complex during CPC specification and differentiation. Genetic knockout studies of individual SWI/SNF subunits together with biochemical purification technologies will continue to provide insight on the functions of each subunits and how that particular subunit mediates the proper assembly and function of the whole complex. These studies may also reveal possible auto feedback regulations within the complex. Moreover, inducible over-expression of SWI/SNF components in vitro and in vivo will likely identify the key components in cSWI/SNF that drive CPC differentiation.

INTERACTION OF SWI/SNF WITH TRANSCRIPTION FACTORS

Numerous studies have established that the interactions of SWI/SNF with key transcription factors are essential for ES cell and organ development. In ESCs, SWI/SNF colocalizes with Oct4, Sox2, and Nanog in a genome-wide manner and functionally interacts with Oct4 and Sox2 to control the target genes for stem cell self-renewal and pluripotency [Ho et al., 2009]. Moreover, Brg1 is shown to promote oligodendrocyte lineage progression and maturation [Yu et al., 2013]. During this process, the interaction of Olig2 with Brg1 pre-patterned the recruitment of Brg1 to oligodendrocyte enhancers. During heart development, SWI/SNF also interacts with GATA4 and TBX5 to promote cardiac differentiation [Lickert et al., 2004]. A detailed genome-wide co-occupancy analysis of cardiac transcription factors with SWI/SNF complex in different cardiac lineages will help identify novel key transcription factors associated with SWI/SNF and may also help characterize heart-specific enhancers and reveal the transcription cascade during CPC differentiation. In addition, biochemical and proteomic studies could also provide information of SWI/SNF partners during development (Fig. 3A).

SYNERGY BETWEEN SWI/SNF AND OTHER EPIGENETIC FACTORS

A synergistic interplay between SWI/SNF and numerous other epigenetic factors are also found essential for development. Brg1 has

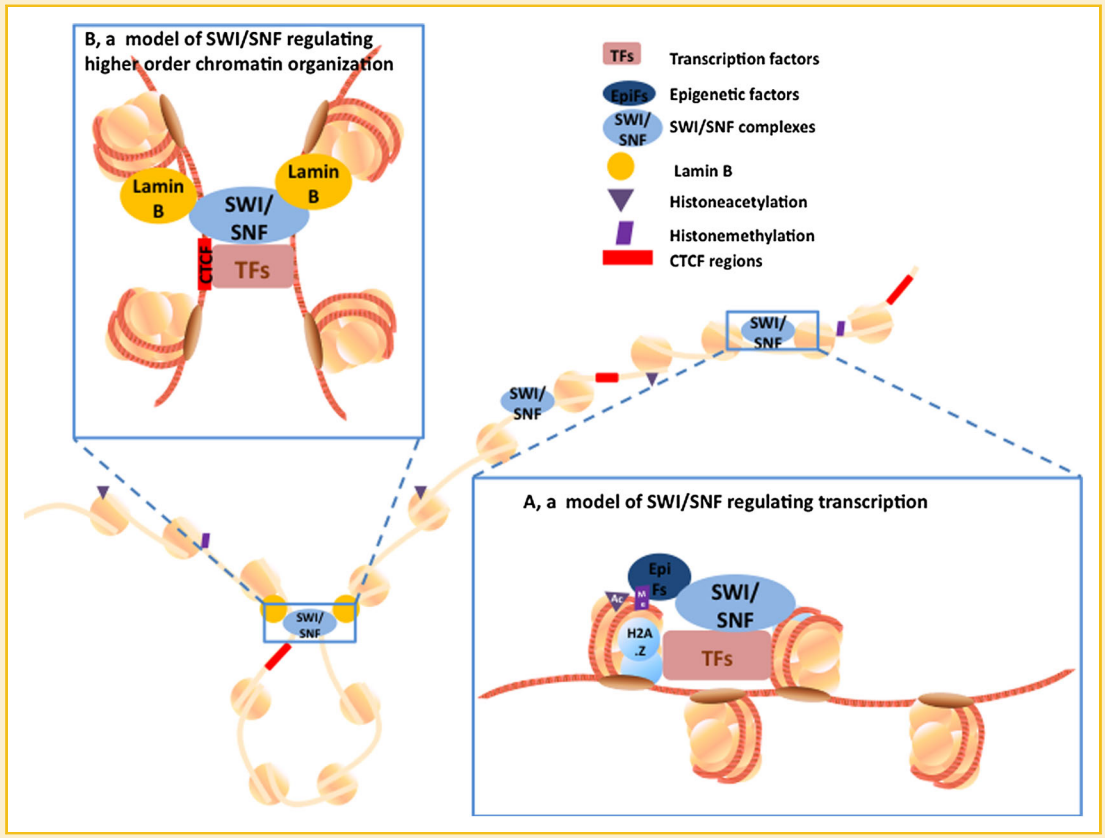

Fig. 3. Potential synergistic functions of SWI/SNF with transcription factors (TFs) and epigenetic factors (EpiFs) during CPC differentiation. A: SWI/SNF remodel nucleosome positions and other chromatin structures to modulate DNA accessibilities, thereby regulating gene expression. B: SWI/SNF could also work with other EpiFs to modulate higher chromatin structure in large scale to regulate long distance DNA–DNA interactions.

been shown to interact with HDACs to direct myocardium maturation [Hang et al., 2010]. Brg1 coupled with histone modifying enzymes also controls expression of critical regulators for oligodendrocyte and muscle differentiation [Dacwag et al., 2007; Yu et al., 2013]. BAF45c could regulate binding of acetylated histone to regulate heart development [Zeng et al., 2010]. Moreover, SWI/SNF antagonizes the role of polycomb groups in many development events. These results suggest that SWI/SNF and related epigenetic factors may form comprehensive networks during development (Fig. 3A). Genome-wide analysis of chromatin modifications with large-scale protein-protein interaction and co-occupancy studies of SWI/SNF and other epigenetic factors in CPC systems developed in vitro will likely identify an integrated chromatin modifying complex network guiding the proper CPC differentiation.

A POSSIBLE PRIMING ROLE OF SWI/SNF FOR CPC DIFFERENTIATION

One intriguing question is whether SWI/SNF plays a priming role to facilitate CPC differentiation. It has been shown that epigentic modifications at chromatin can prime the target genes for expressions. These genes are primed at a "poised" status–silent but will be turned on immediately upon receiving signal for lineage committment. The most prominent modifications are the concurrent presence of H3K4me3 and H3K27me3 at gene promoters and enhancers, termed bivalent domains. In ESCs, many poised genes encode key factors for lineage differentiation [Mikkelsen et al., 2007]. These genes are also marked by Brg1 and low nucleosomal density [Rada-Iglesias et al., 2011].

During CPC differentiation, from ES cells to mesodermal cells, then to cardiac progenitor cells (CPCs), and finally to cardiomyocytes, there are unique epigenetic signatures in different stages. miRNA, lncRNA, and histone modifications coordinate with specific gene expression at different cell types [Paige et al., 2012; Wamstad et al., 2012]. These studies indicate that the epigenetic profiles define the distant enhancer elements that is required for cardiac development. Moreover, the pattern of histone modification and CTCF-binding at enhancer elements are highly associated with cell type specific gene expression [Heintzman et al., 2009].

In addition to histone modifications, nucleosome positioning are shown to associated with active and inactive genes [Schones et al., 2008]. Several studies have suggested that SWI/SNF mediated nucleosome remodeling have a priming role in specifying gene expression during development. For example, nucleosome-depleted regions at transcription start site and transcription termination sites are associated with active genes [Li et al., 2012; Hu et al., 2013]. H2A.Z is more abundant in active gene in various organisms. During mouse ES cell differentiation, H2A.Z regulates gene expression by

mediating -1 nucleosome depletion. H2A.Z is essential for the recruitment of SWI/SNF to yeast. In mammals, a recent study just showed that SWI/SNF could be recruited by H2A.Z to deplete nucleosome during differentiation [Li et al., 2012]. Binding of RARα was significantly compromized genome-wide by H2A.Z knockdown [Hu et al., 2013]. Our previous research imply that BAF180/PBAF plays a role in RA signaling during heart development, [Huang et al., 2008]. Therefore, RXRα and H2A.Z together may recruit BAF180 and further recruit nucleosome disassembly complexes SWI/SNF. It is possible that this preference have impact on pre-patterning chromatin signatures. A detailed nucleosome and histone modification mapping in both wild type and SWI/SNF knockout cells combined with gene expression study at different cardiac lineages will define whether SWI/SNF mediated nucleosome remodeling play a key priming role for CPC differentiation (Fig. 3A).

Not only nucleosome positioning in a single gene could prime its expression, large chromatin domains could also have an impact on gene expression. SWI/SNF were found to co-localize with CTCF and lamin B at many long-distance DNA–DNA interaction regions [Euskirchen et al., 2011]. Several higher order structures are reported recently including topological domains [Dixon et al., 2012], A and B compartments [Ryba et al., 2010], lamina-associated domains (LADs) [Peric-Hupkes et al., 2010], replication time zones [Hiratani et al., 2010], and large organized chromatin K9 modification (LOCK) domains [Wen et al., 2009]. Boundaries of topological domains are enriched for the insulator binding protein CTCF and LADs are related with lamin B. The big question to address next is whether SWI/SNF contributes to form or break these structures. SHPRH is a member of the SWI/SNF family of ATPases/helicases, SHPRH contains the PHD (Plant HomeoDomain) domain that can interact with dimethylated histone H3 at K9 (H3K9Me2) in vitro, implying that SWI/SNF maybe able to break the LOCK domains. However, the relationship of higher order chromatin structures and their regulation are poorly defined at the moment. A detailed analysis of higher order chromatin structure changes in SWI/SNF mutants along with the studies of the function and regulation of higher order chromatin structures will further advance our knowledge of SWI/SNF in epigenetic regulation during CPC differentiation (Fig. 3B).

Detailed analyses of SWI/SNF complexes during mammalian development will establish a series of integrated and dynamic chromatin- and histone-modifying events and transcription networks for proper CPC differentiation. These studies may also provide important clues in heart tissue de-differentiation or directed reprogramming of one mature cell type into another in the heart. Knowledge gained from future studies of SWI/SNF in CPC differentiation may provide novel targets to help develop small molecules, gene therapy, and cell based therapies for heart disease.

ACKNOWLEDGMENTS

We thank the members of the Wang laboratory for helpful discussions of the manuscript. Z. W. is supported by the US National Institutes of Health grant R01 HL109054-01 and M. H. S. is supported by the Stem Cell and Regenerative Medicine Consortium at the University of Hong Kong.

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