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BET-ting on Chromatin-Based Therapeutics for Heart Failure

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Abstract

Studies of transcriptional mechanisms in heart failure have focused heavily on roles of sequence-specific DNA-binding factors such as NFAT, MEF2 and GATA4. Recent findings have illuminated crucial functions for epigenetic regulators in the control of cardiac structural remodeling and mechanical dysfunction in response to pathological stress. Here, we review the current understanding of chromatin-dependent signal transduction in cardiac gene control, and highlight potential for pharmacologic regulation of BET acetyl-lysine binding proteins as a means of treating heart failure.

1. Introduction

Abnormalities in the control of gene expression are central to the pathogenesis of heart failure (HF). Transcript expression profiling in animal models [1] and diseased human hearts [2] consistently demonstrates stereotypical patterns of aberrant myocardial gene control. The most cogent evidence that implicates transcriptional misregulation in HF pathogenesis comes from a large body of work using murine gene-targeting and transgenesis.

Collectively, these studies have clearly demonstrated that activation of specific DNA-binding transcription factors (TFs), such as NFAT, MEF2, NF- κ B, GATA4, and C-MYC, is critical for pathological cardiac remodeling *in vivo* [3]. However, the precise molecular mechanisms by which these potent TFs signal downstream to trigger pathologic gene expression in the heart has remained poorly understood. To unravel these mechanisms, one must consider that TFs function in the context of chromatin to drive cell state-specific gene expression programs [4]. In this article, we review current concepts in eukaryotic transcription, and highlight recent studies that explore the role of chromatin-dependent signal transduction in cardiac gene control and HF pathogenesis. As drugs that target chromatin-dependent signaling effectors are being developed as anti-cancer agents [5], a

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deeper understanding of these epigenetic pathways in the myocardium may provide novel therapeutic opportunities.

2. Current Concepts in Eukaryotic Gene Control

Chromatin refers to a dynamic macromolecular complex of genomic DNA complexed with a diverse array of RNA and proteins [6]. The fundamental unit of chromatin is the nucleosome, comprised of 147 base pairs of double-stranded DNA wrapped in approximately 1.7 superhelical turns around a histone octamer consisting of two copies each of the core histones H2A, H2B, H3 and H4 [7]. Histones within nucleosomes can be post-translationally modified and/or exchanged with variants to alter primary chromatin structure [8]. Primary chromatin, in turn, is arrayed into higher order three-dimensional configurations that permit local accessibility of the genome and participate in signaling. By vastly expanding the signaling repertoire of the primary DNA template, higher order chromatin structure endows eukaryotes with the ability to generate remarkable cellular plasticity from a single genome [4, 9].

We will first briefly review some fundamental features of eukaryotic gene regulation, as these concepts are the necessary framework for understanding cardiac gene control in physiology and disease. Eukaryotic cell identity or more broadly, “cellular state”, is largely governed by precise spatiotemporal coordination of gene expression programs [4]. While the concept of “cell state transformation” is obviously pertinent to the study of organogenesis and developmental specification (e.g., the differentiation of a pluripotent stem cell into a cardiomyocyte), we emphasize here that activation of pathologic transcriptional programs in the stressed heart (e.g., transformation of a healthy cardiomyocyte into one that is hypertrophied and hypo-contractile) also represents an equally robust cell state transition that is driven by defined molecular events. Control of these gene expression programs is orchestrated by dynamic interplay between activity of DNA-binding TFs and changes in higher-order chromatin structure. Accumulating evidence demonstrates that a limited number of TFs are capable of controlling the selective transcription of genes by RNA Polymerase II (Pol II), thereby governing any given cell state [4]. TFs typically regulate gene expression by binding regulatory DNA elements called enhancers, an event which recruits cofactors and facilitates assembly of the general transcriptional machinery (e.g. Pol II) to the transcriptional start sites of target genes [10, 11]. An active enhancer typically binds multiple TFs in a cooperative fashion and regulates transcription from core promoters, often via long-range genomic interactions that involve looping of DNA [12, 13]. In addition, TFs can also bind to core promoter elements in proximity to transcriptional start sites to recruit transcriptional machinery and regulate cellular state [14].

A critical mechanism by which enhancer-bound TFs set the stage for gene control is via the recruitment of co-factors that alter local chromatin structure. Two major categories of cofactors are those that mobilize nucleosomes (e.g. the ATP-dependent chromatin remodeling complexes) [6] and those that enzymatically modify histones via post-translational modifications (e.g. acetylation, methylation, phosphorylation, and ubiquitylation) [15]. With regard to the latter, there are enzymes that add or remove post-translational modifications, which have been dubbed epigenetic “writers” and “erasers”,

respectively. Consequently, there are proteins harboring recognition motifs for each of these histone modifications, termed epigenetic “readers”, which facilitate protein complex formation and signal propagation. Together, these modifications to DNA and DNA-associated proteins alter local chromatin structure in a stereotypical fashion across the regulatory and coding regions of the genome in a manner that correlates with transcriptional activity [4, 15]. For example, H3K27ac is found at active promoters and enhancers, H3K36me3 marks actively transcribed gene bodies, and H3K27me3 marks heterochromatic or transcriptionally repressed regions [16]. In addition to histone proteins, DNA itself can be covalently modified, including methylation of carbon-5 of cytosine (5mC), a mark that has been associated with transcriptional repression [16]. Collectively, the dynamic interplay between TFs and alterations in local chromatin structure dictate higher order genomic architecture and allow genomic signal transduction to occur with spatiotemporal precision [4, 9].

The main cellular machinery for transcribing protein-coding genes is the Pol II complex. Phases of Pol II-dependent transcription include formation of initiation complexes at transcriptional start sites, promoter-proximal pausing, pause release, elongation and termination. The dynamics of Pol II function are tightly regulated in a locus-specific and signal-dependent manner. In general, TFs and recruited co-regulatory proteins signal via chromatin to control both initiation and elongation of Pol II [17–19]. Once a recruited Pol II molecule initiates transcription, it generally travels a short distance (generating a nascent mRNA of 20–50 nucleotides), and then pauses. Pausing is, in part, mediated by the physical association of Pol II with pause control factors such as NELF and DSIF [17]. Release of this paused state and subsequent transcriptional elongation is mediated by recruitment and activation of kinase complexes, such as positive transcription elongation factor b (P-TEFb). P-TEFb phosphorylates Pol II and its associated pausing factors, thereby allowing pause release and productive transcriptional elongation [20]. It is now increasingly appreciated that a major mechanism by which many transcription factors affect gene expression is via downstream control of pause release and transcriptional elongation. Factors that regulate this latter step of pause release can have profound effects on cell state [4], some of which have been shown to be critically involved in pathologic cardiac remodeling [21, 22] (see section 5 below).

3. Probing the Epigenome Using Rapidly Evolving Technologies

The last decade has witnessed unprecedented growth in epigenomic technologies. Rapidly evolving technical advances in our ability to probe TF enrichment, histone modifications, chromatin structure, and transcriptional dynamics on a genome-wide scale has transformed our understanding of how cell state-specific gene expression is orchestrated. Previous to the advent of next generation sequencing (NGS), assays of chromatin structure (e.g., chromatin immunoprecipitation [ChIP] and 5mC detection) were used to specifically probe loci of interest one at a time. However, NGS platforms now allow for massively parallel short reads of DNA sequence in an increasingly high-throughput, fast, and cost effective manner [16]. Therefore, the epigenetic assays previously used to measure a single locus are now integrated with NGS technology to provide unbiased, genome-wide chromatin state maps and transcript expression profiles. These state maps include genome-wide assessment of

nucleosome structure, histone modifications, DNA modifications, transcription factor cistromes, enrichment of chromatin-associated proteins and nucleic acids, long-range chromatin interactions, and transcriptional dynamics [16, 23]. Much of our knowledge of chromatin state across a wide variety of cell and tissue types has come from the NIH/NHGRI sponsored ENCODE consortium (Encyclopedia of DNA Elements). Data from ENCODE has significantly advanced the identification of functional elements within the human genome and has provided essential insight into cell-state specific gene control [24]. The elucidation of thousands of previously unappreciated regulatory elements (e.g. enhancers) and non-coding transcripts (e.g. lnc-RNAs) is rapidly changing our understanding of cellular differentiation, development and disease pathogenesis [16]. As human genome-wide association studies have demonstrated that the vast majority of disease-associated loci map to non-protein coding regions of DNA [25], the mechanistic insight gleaned from epigenomic maps are likely to provide a more detailed understanding of disease susceptibility and pathogenesis. These genome-wide assays of chromatin state, which are just beginning to be applied to cardiovascular biology [21, 26, 27], have the potential to transform our understanding of cardiac hypertrophy and HF.

4. ATP-Dependent Chromatin Remodeling Complexes in Cardiac Hypertrophy

Chromatin remodeling complexes (CRCs) harness the energy liberated by ATP hydrolysis to disrupt the DNA-nucleosome electrostatic association, thereby shuttling nucleosomes along DNA, exchanging or removing nucleosomes, and altering genome accessibility [6, 28]. CRCs are large macromolecular aggregates consisting of 9–12 catalytic and regulatory subunits [29]. The catalytic domains have ATPase activity while regulatory domains have a variety of functions, including “reading” covalently modified histones, modulating ATPase activity, and interacting with other chromatin-associated proteins [29]. There are four major human CRC families (the SWI/SNF, ISWI, CHD and INO80) that can catalyze a broad range of structural changes to chromatin [28, 30]. Of these families, the role of the SWI/SNF complexes in cardiac development and hypertrophy has been the focus of detailed investigation [29, 31]. BRG1 (SMARCA4) is the essential ATPase subunit of the BAF complex (the mammalian analog of the yeast SWI/SNF complex). Studies in conditional *Brg1*-deficient mice show that BRG1 promotes myocyte proliferation by maintaining *Bmp10* expression and suppressing *p57^{kip2}* expression. BRG1 is essential for preserving the fetal cardiomyocyte gene expression program, as evidenced by its ability to activate the fetal isoform of myosin heavy chain (*Myh7*/β-MyHC) and repress the adult isoform (*Myh6*/α-MyHC) [31]. *Brg1* expression is turned off in adult cardiomyocytes, but re-induced after pressure-overload in mice [31]. Furthermore, in patients with hypertrophic cardiomyopathy (HCM), BRG1 expression is significantly increased over that of control hearts, and higher BRG1 levels correlate with HCM disease severity and the extent of MyHC isoform switching [31]. Mechanistic studies reveal that BRG1 regulates the fetal cardiomyocyte state, in part, via participating in dynamic chromatin complexes with histone deacetylases (HDACs) and poly (ADP ribose) polymerase (PARP). In the adult, re-activation of BRG1 and its interactions with HDACs and PARP are required for MyHC isoform switching and cardiac hypertrophy. Based on these data, BRG1 can be considered to be a positive regulator

of fetal gene expression and cardiac hypertrophy [31]. Genome-wide studies detailing BRG1 enrichment in the heart during pathological stress and BRG1-dependent gene expression will be informative, particularly to assess nucleosome remodeling at critical enhancer loci [32].

5. Regulation of Cardiac Hypertrophy by P-TEFb

As noted previously, P-TEFb plays a critical role in gene regulation by stimulating release of paused, promoter-proximal Pol II, with subsequent transcriptional elongation [20]. Active P-TEFb consists of a complex of cyclin-dependent kinase 9 (CDK9) and cyclin T1 or cyclin T2. CDK9-mediated phosphorylation of serine-2 of the heptad repeat in the carboxy-terminal domain (CTD) of Pol II, as well as of pause-control factors such as NELF and DSIF, triggers pause release and productive transcriptional elongation. A significant portion of cellular P-TEFb also exists in an inactive state when bound to a complex containing the 330-nucleotide small nuclear RNA, 7SK, and hexamethylene bis-acetamide inducible 1 (HEXIM1), which is also known as cardiac lineage protein (CLP) (Figure 1).

Pioneering work by the Schneider group clearly demonstrated a crucial role for CDK9-dependent phosphorylation of Pol II in the control of cardiomyocyte hypertrophy [22, 33]. Activation of P-TEFb/CDK9 in response to hypertrophic stimuli was shown to involve its dissociation of 7SK from the complex. Furthermore, cardiomyocyte-specific overexpression of Cyclin T1 in mice was sufficient to drive pathologic cardiac hypertrophy *in vivo* [22]. Consistent with these findings, several subsequent studies by Siddiqui and colleagues revealed a role for HEXIM1 as a negative regulator of cardiac hypertrophy. When bound to 7SK, HEXIM1 potently represses CDK9 catalytic activity [34]. Global deletion of *Hexim1* in mice resulted in cardiac developmental defects and embryonic lethality [31]; mice harboring an insertional mutation that results in disruption of the carboxy-terminus of HEXIM1 also exhibited defects in cardiac development [35]. The absence of HEXIM1 in the embryonic heart led to elevated expression of genes associated with pathological hypertrophy, including *atrial natriuretic factor (Nppa)* and *β -myosin heavy chain (Myh7)*, suggesting a role for HEXIM1 as an endogenous inhibitor of hypertrophy. Indeed, ensuing work demonstrated that hypertrophic signals cause dissociation of HEXIM1 from the P-TEFb complex [36, 37], and adult mice lacking a single copy of the *Hexim1* gene develop exaggerated cardiac hypertrophy and fibrosis in response to CDK9 activation (via CyclinT1 overexpression) [37] or angiotensin II infusion [38].

Effects of ectopically expressing HEXIM1 in the heart appear to differ depending on the transgenic approach that is employed. Transgenic overexpression of HEXIM1 in the heart using a constitutive α -MyHC promoter led to inhibition of pulmonary hypertension-induced right ventricular hypertrophy [39]. In contrast, inducible cardiac-specific expression of ectopic HEXIM1 in adult mice led to a growth response resembling some features of physiologic hypertrophy, with increased cardiac vascularization and ventricular performance, and without induction of natriuretic peptide genes [40]. As these mice also displayed sinus bradycardia and QT prolongation, it will be important to determine whether long-term activation HEXIM1 is arrhythmogenic or cardiotoxic. Furthermore, it will be interesting to assess whether these mice are resistant pathological stress, such as pressure-overload and myocardial infarction, and whether the mice exhibit reduced cardiac fibrosis

compared to wild-type animals, since fibrosis is usually only associated with pathological hypertrophy.

Another component of the P-TEFb complex was recently identified and shown to impact cardiac gene expression. Overexpression studies revealed that Coup-TF interacting protein-2 (CTIP2) is capable of associating with HEXIM1/7SK and inhibiting CDK9 catalytic activity, and ChIP experiments suggested that CTIP2 associates with regulatory elements in the β -MyHC gene [41]. Further evaluation of the role of endogenous CTIP2 in the control of P-TEFb and cardiac hypertrophy is warranted.

6. Novel Functions for BET Acetyl-Lysine Readers in the Control of Cardiac Hypertrophy

We recently discovered that recruitment of P-TEFb to target genes in response to pathological stress in the heart is governed by bromodomain and extraterminal (BET) acetyl-lysine reader proteins [21, 42]. The BET family consists of four family members (BRD2, BRD3, BRD4 and the testis-specific BRDT) all of which have two tandem N-terminal acetyl-lysine recognition motifs, or bromodomains. Of the BETs, BRD4 is the most extensively characterized and is the family member that has been shown to specifically interact with the P-TEFb complex. BRD4 associates with acetylated nucleosomal histones via its bromodomains and activates CDK9 activity at transcriptional start sites through a carboxy-terminal P-TEFb-interacting domain [43–45]. As such, BRD4 functions to promote transcriptional elongation by triggering phosphorylation of the Pol II CTD (Figure 1).

BRD4 has mostly been studied in the context of cell cycle gene regulation, and is a promising target for cancer therapy [5]. Remarkably, neither BRD4 nor other BET proteins had previously been studied in the heart. Mice harboring systemic deletion or even haploinsufficiency of *Brd2* or *Brd4* have severe multisystem developmental abnormalities [46], and mice harboring conditional BET alleles have not been created to date. We found that endogenous BRD4 protein expression is induced in the heart during cardiac hypertrophy, and BRD4 is recruited to a select set of genes in response to hypertrophic stimuli. Gene expression profiling and genome-wide ChIP-sequencing studies revealed that BRD4 promotes transcriptional pause release of Pol II in response to pathological stress in the heart, which is consistent with its ability to associate with P-TEFb. Our data also demonstrated that BRD4 enriches at active cardiac enhancers and signals downstream to Pol II, thereby integrating the transcriptional output of pro-hypertrophic TFs such as NFAT and GATA4. Therefore, BRD4 bridges upstream signaling cascades, pro-hypertrophic TFs and chromatin to drive expression of the gene program for cardiac hypertrophy. Together, these studies defined BRD4 as a nodal effector of pathological cardiac remodeling.

Since a multitude of redundant signaling networks are capable of promoting pathological cardiac hypertrophy [3], it has long been hypothesized that the most effective therapeutic strategy would target a distal signaling integrator such as BRD4. To begin to address the potential of BRD4 as a target in HF, studies were performed with a highly selective and potent small molecule BET inhibitor, JQ1. JQ1, which was developed by the Bradner laboratory, works by competitively binding the BET bromodomains and displacing these

acetyl-lysine readers from chromatin, resulting in suppression of downstream signaling events to Pol II [47]. Remarkably, in a mouse pressure-overload model, early administration of JQ1 dramatically blocked the development of left ventricular hypertrophy, interstitial fibrosis and systolic dysfunction [21, 42]. JQ1 was well tolerated and did not reduce endurance exercise capacity in mice. Based on these findings in murine models, additional pre-clinical studies are clearly needed to further validate BRD4 as a therapeutic target for HF.

While BRD4 is globally enriched at active cardiac enhancers, this protein regulates specific gene expression programs [21, 42]. We note that other ubiquitously enriched chromatin regulators, such as the mediator subunit MED13 [48] and the pause control factor NELF-B [49], also control specific gene expression programs in the heart. Therefore, understanding how these seemingly omnipresent chromatin regulators exert highly selective gene control in a stimulus-coupled manner remains a central question in cardiac epigenetics. Future studies on BET proteins also need to address the mechanisms controlling BRD4 protein induction during cardiac hypertrophy, and the molecular basis by which upstream signaling cascades control recruitment of BRD4 to select gene regulatory elements. Furthermore, since HDACs and histone acetyltransferases serve integral functions in the control cardiac hypertrophy [50], it will be of great interest to assess the extent to which these histone modifying enzymes crosstalk with BRD4 to regulate pathological gene expression in the heart. In addition to BRD4, BRD2 and BRD3 are also expressed in the heart, yet nothing is known about the individual functions of these BET family members in cardiac muscle, highlighting a rich area for future investigation. The creation of mice harboring conditional BET alleles will be essential to dissect the gene and tissue-specific roles of the BETs in cardiac biology. With regard to translating pre-clinical findings into clinical trials for HF, the therapeutic benefit of any pharmacologic agent must be carefully weighed against the potential risk of toxicity, especially for emerging classes of drugs such as BET inhibitors. However, it should be noted that the feasibility of safely inhibiting BET proteins in humans was established with RVX-208 in patients with atherosclerosis [51], and several novel BET inhibitors that are in clinical trials for cancer [52]. Additional experiments that test the efficacy of BET inhibitors in pre-clinical models of HF are clearly justified in order to address several key questions, including the following: (i) is BET inhibition capable of reversing established cardiac dysfunction?, (ii) does BET inhibition block ischemia-induced cardiac dysfunction?, (iii) is BET inhibition efficacious in large animal models of HF?, and (iv) does BET inhibition have added benefit beyond current pharmacotherapies for HF (e.g., β_1 -adrenergic receptor blockade and inhibition of the renin-angiotensin system)?

7. Conclusions

Research on chromatin regulation in the heart is still in its infancy. With the advent of novel epigenomic technologies, we are likely to see dramatic and rapid advances in this field in the near future. Our ability to define novel regulatory elements, such as enhancers, in the developing and diseased heart represents a particularly exciting area of investigation. As the vast majority of disease associated polymorphisms discovered in human genome-wide association studies do not map to protein coding regions [25], it is possible that many such loci regulate chromatin structure or non-protein coding transcripts (e.g. lncRNAs). Our

ability to probe the epigenome, when coupled to rapidly evolving technology for genome editing, has the potential to rapidly define function of these regulatory genomic elements. In addition to understanding disease pathogenesis, our ability to pharmacologically manipulate many chromatin regulators, as exemplified by HDAC and BET inhibition, opens the door for innovative “epigenetic therapies” for common and lethal diseases such as human HF.

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References

1. Zhao M, Chow A, Powers J, Fajardo G, Bernstein D. Microarray analysis of gene expression after transverse aortic constriction in mice. *Physiol Genomics*. 2004; 19:93–105. [PubMed: 15292486]
2. Hannenhalli S, Putt ME, Gilmore JM, Wang J, Parmacek MS, Epstein JA, et al. Transcriptional genomics associates FOX transcription factors with human heart failure. *Circulation*. 2006; 114:1269–1276. [PubMed: 16952980]
3. van Berlo JH, Maillet M, Molken JD. Signaling effectors underlying pathologic growth and remodeling of the heart. *J Clin Invest*. 2013; 123:37–45. [PubMed: 23281408]
4. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. *Cell*. 2013; 152:1237–1251. [PubMed: 23498934]
5. Helin K, Dhanak D. Chromatin proteins and modifications as drug targets. *Nature*. 2013; 502:480–488. [PubMed: 24153301]
6. Hargreaves DC, Crabtree GR. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res*. 2011; 21:396–420. [PubMed: 21358755]
7. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 1997; 389:251–260. [PubMed: 9305837]
8. Zhou VW, Goren A, Bernstein BE. Charting histone modifications and the functional organization of mammalian genomes. *Nat Rev Genet*. 2011; 12:7–18. [PubMed: 21116306]
9. Schreiber SL, Bernstein BE. Signaling network model of chromatin. *Cell*. 2002; 111:771–778. [PubMed: 12526804]
10. Ong CT, Corces VG. Enhancers: emerging roles in cell fate specification. *EMBO Rep*. 2012; 13:423–430. [PubMed: 22491032]
11. Spitz F, Furlong EE. Transcription factors: from enhancer binding to developmental control. *Nat Rev Genet*. 2012; 13:613–626. [PubMed: 22868264]
12. Krivega I, Dean A. Enhancer and promoter interactions-long distance calls. *Curr Opin Genet Dev*. 2012; 22:79–85. [PubMed: 22169023]
13. Panne D. The enhanceosome. *Curr Opin Struct Biol*. 2008; 18:236–242. [PubMed: 18206362]
14. Goodrich JA, Tjian R. Unexpected roles for core promoter recognition factors in cell-type-specific transcription and gene regulation. *Nat Rev Genet*. 2010; 11:549–558. [PubMed: 20628347]
15. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011; 21:381–395. [PubMed: 21321607]
16. Rivera CM, Ren B. Mapping human epigenomes. *Cell*. 2013; 155:39–55. [PubMed: 24074860]
17. Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet*. 2012; 13:720–731. [PubMed: 22986266]
18. Fuda NJ, Ardehali MB, Lis JT. Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature*. 2009; 461:186–192. [PubMed: 19741698]
19. Malik S, Roeder RG. The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. *Nat Rev Genet*. 2010; 11:761–772. [PubMed: 20940737]

20. Zhou Q, Li T, Price DH. RNA polymerase II elongation control. *Annu Rev Biochem.* 2012; 81:119–143. [PubMed: 22404626]
21. Anand P, Brown JD, Lin CY, Qi J, Zhang R, Artero PC, et al. BET bromodomains mediate transcriptional pause release in heart failure. *Cell.* 2013; 154:569–582. [PubMed: 23911322]
22. Sano M, Abdellatif M, Oh H, Xie M, Bagella L, Giordano A, et al. Activation and function of cyclin T-Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. *Nat Med.* 2002; 8:1310–1317. [PubMed: 12368904]
23. Kwak H, Fuda NJ, Core LJ, Lis JT. Precise maps of RNA polymerase reveal how promoters direct initiation and pausing. *Science.* 2013; 339:950–953. [PubMed: 23430654]
24. Qu H, Fang X. A brief review on the Human Encyclopedia of DNA Elements (ENCODE) project. *Genomics Proteomics Bioinformatics.* 2013; 11:135–141. [PubMed: 23722115]
25. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science.* 2012; 337:1190–1195. [PubMed: 22955828]
26. Papait R, Cattaneo P, Kunderfranco P, Greco C, Carullo P, Guffanti A, et al. Genome-wide analysis of histone marks identifying an epigenetic signature of promoters and enhancers underlying cardiac hypertrophy. *Proc Natl Acad Sci U S A.* 2013; 110:20164–20169. [PubMed: 24284169]
27. Wamstad JA, Alexander JM, Truty RM, Shrikumar A, Li F, Eilertson KE, et al. Dynamic and coordinated epigenetic regulation of developmental transitions in the cardiac lineage. *Cell.* 2012; 151:206–220. [PubMed: 22981692]
28. Narlikar GJ, Sundaramoorthy R, Owen-Hughes T. Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. *Cell.* 2013; 154:490–503. [PubMed: 23911317]
29. Chang CP, Bruneau BG. Epigenetics and cardiovascular development. *Annu Rev Physiol.* 2012; 74:41–68. [PubMed: 22035349]
30. Puri PL, Mercola M. BAF60 A, B, and Cs of muscle determination and renewal. *Genes Dev.* 2012; 26:2673–2683. [PubMed: 23222103]
31. Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, et al. Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature.* 2010; 466:62–67. [PubMed: 20596014]
32. Hu G, Schones DE, Cui K, Ybarra R, Northrup D, Tang Q, et al. Regulation of nucleosome landscape and transcription factor targeting at tissue-specific enhancers by BRG1. *Genome Res.* 2011; 21:1650–1658. [PubMed: 21795385]
33. Sano M, Schneider MD. Cyclin-dependent kinase-9: an RNAPII kinase at the nexus of cardiac growth and death cascades. *Circ Res.* 2004; 95:867–876. [PubMed: 15514168]
34. Michels AA, Fraldi A, Li Q, Adamson TE, Bonnet F, Nguyen VT, et al. Binding of the 7SK snRNA turns the HEXIM1 protein into a P-TEFb (CDK9/cyclin T) inhibitor. *EMBO J.* 2004; 23:2608–2619. [PubMed: 15201869]
35. Montano MM, Doughman YQ, Deng H, Chaplin L, Yang J, Wang N, et al. Mutation of the HEXIM1 gene results in defects during heart and vascular development partly through downregulation of vascular endothelial growth factor. *Circ Res.* 2008; 102:415–422. [PubMed: 18079413]
36. Espinoza-Derout J, Wagner M, Shahmiri K, Mascareno E, Chaqour B, Siddiqui MA. Pivotal role of cardiac lineage protein-1 (CLP-1) in transcriptional elongation factor P-TEFb complex formation in cardiac hypertrophy. *Cardiovasc Res.* 2007; 75:129–138. [PubMed: 17459355]
37. Espinoza-Derout J, Wagner M, Saliccioli L, Lazar JM, Bhaduri S, Mascareno E, et al. Positive transcription elongation factor b activity in compensatory myocardial hypertrophy is regulated by cardiac lineage protein-1. *Circ Res.* 2009; 104:1347–1354. [PubMed: 19443839]
38. Mascareno E, Galatioto J, Rozenberg I, Saliccioli L, Kamran H, Lazar JM, et al. Cardiac lineage protein-1 (CLP-1) regulates cardiac remodeling via transcriptional modulation of diverse hypertrophic and fibrotic responses and angiotensin II-transforming growth factor beta (TGF-beta1) signaling axis. *J Biol Chem.* 2012; 287:13084–13093. [PubMed: 22308025]
39. Yoshikawa N, Shimizu N, Maruyama T, Sano M, Matsuhashi T, Fukuda K, et al. Cardiomyocyte-specific overexpression of HEXIM1 prevents right ventricular hypertrophy in hypoxia-induced pulmonary hypertension in mice. *PLoS One.* 2012; 7:e52522. [PubMed: 23300697]

40. Montano MM, Desjardins CL, Doughman YQ, Hsieh YH, Hu Y, Bensinger HM, et al. Inducible re-expression of HEXIM1 causes physiological cardiac hypertrophy in the adult mouse. *Cardiovasc Res.* 2013; 99:74–82. [PubMed: 23585471]
41. Cherrier T, Le D, V, Eilebrecht S, Riclet R, Marban C, Dequiedt F, et al. CTIP2 is a negative regulator of P-TEFb. *Proc Natl Acad Sci U S A.* 2013; 110:12655–12660. [PubMed: 23852730]
42. Spiltoir JI, Stratton MS, Cavaasin MA, Demos-Davies K, Reid BG, Qi J, et al. BET acetyllysine binding proteins control pathological cardiac hypertrophy. *J Mol Cell Cardiol.* 2013; 63:175–179. [PubMed: 23939492]
43. Bisgrove DA, Mahmoudi T, Henklein P, Verdin E. Conserved P-TEFb-interacting domain of BRD4 inhibits HIV transcription. *Proc Natl Acad Sci U S A.* 2007; 104:13690–13695. [PubMed: 17690245]
44. Jang MK, Mochizuki K, Zhou M, Jeong HS, Brady JN, Ozato K. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Mol Cell.* 2005; 19:523–534. [PubMed: 16109376]
45. Yang Z, Yik JH, Chen R, He N, Jang MK, Ozato K, et al. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. *Mol Cell.* 2005; 19:535–545. [PubMed: 16109377]
46. Houzelstein D, Bullock SL, Lynch DE, Grigorieva EF, Wilson VA, Beddington RS. Growth and early postimplantation defects in mice deficient for the bromodomain-containing protein Brd4. *Mol Cell Biol.* 2002; 22:3794–3802. [PubMed: 11997514]
47. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, et al. Selective inhibition of BET bromodomains. *Nature.* 2010; 468:1067–1073. [PubMed: 20871596]
48. Grueter CE, van RE, Johnson BA, DeLeon SM, Sutherland LB, Qi X, et al. A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. *Cell.* 2012; 149:671–683. [PubMed: 22541436]
49. Pan H, Qin K, Guo Z, Ma Y, April C, Gao X, et al. Negative Elongation Factor Controls Energy Homeostasis in Cardiomyocytes. *Cell Rep.* 2014
50. McKinsey TA. Therapeutic potential for HDAC inhibitors in the heart. *Annu Rev Pharmacol Toxicol.* 2012; 52:303–319. [PubMed: 21942627]
51. Balder JW, Staels B, Kuivenhoven JA. Pharmacological interventions in human HDL metabolism. *Curr Opin Lipidol.* 2013; 24:500–509. [PubMed: 24184942]
52. Wee S, Dhanak D, Li H, Armstrong SA, Copeland RA, Sims R, et al. Targeting epigenetic regulators for cancer therapy. *Ann N Y Acad Sci.* 2014; 1309:30–36. [PubMed: 24571255]

Figure 1. Chromatin-dependent signaling during cardiac hypertrophy via BRD4 and P-TEFb
P-TEFb (CDK9/Cyclin T) stimulates transcriptional elongation through phosphorylation of the CTD of RNA Pol II. In response to stress signals, P-TEFb is activated at pro-hypertrophic gene regulatory elements through association with the enhancer-enriched, acetyl-lysine binding protein BRD4. Pathological cardiac hypertrophy can be blocked with JQ1, a small molecule that prevents binding of BRD4 bromodomains with acetyl-histones, thereby inhibiting activity of P-TEFb at the transcriptional start sites of pro-hypertrophic genes. A complex of HEXIM1, CTIP2 and 7SK RNA provides an endogenous mechanism for inhibition of P-TEFb. The molecular mechanisms that couple stress signaling cascades to recruitment of BRD4 to specific chromatin loci in the heart remain poorly understood.