ROLE OF EPIGENETICS IN CARDIAC DEVELOPMENT AND CONGENITAL DISEASES

Thomas Moore-Morris, Patrick Piet van Vliet, Gregor Andelfinger, and Michel Puceat

Université Aix-Marseille, INSERM UMR-1251, Marseille, France; Cardiovascular Genetics, Department of Pediatrics, CHU Sainte-Justine, Montreal, Quebec, Canada; Université de Montréal, Montreal, Quebec, Canada; and Laboratoire International Associé INSERM, Marseille France-CHU Ste Justine, Quebec, Canada

Moore-Morris T, van Vliet PP, Andelfinger G, Puceat M. Role of Epigenetics in Cardiac Development and Congenital Diseases. Physiol Rev 98: 2453–2475, 2018. Published August 29, 2018; doi:10.1152/physrev.00048.2017.—The heart is the first organ to be functional in the fetus. Heart formation is a complex morphogenetic process regulated by both genetic and epigenetic mechanisms. Congenital heart diseases (CHD) are the most prominent congenital diseases. Genetics is not sufficient to explain these diseases or the impact of them on patients. Epigenetics is more and more emerging as a basis for cardiac malformations. This review brings the essential knowledge on cardiac biology of development. It further provides a broad background on epigenetics with a focus on three-dimensional conformation of chromatin. Then, we summarize the current knowledge of the impact of epigenetic anomalies on cardiac cell fate decision. We further provide an update on the epigenetic anomalies in the genesis of CHD.

I. INTRODUCTION

The heart is the first organ to be functional in the fetus. The most primitive heart in the cephalochordate Amphioxus is just a contractile vessel. During evolution, as organisms grew in size and had to adapt to new environments, the heart had to grow in three dimensions and acquire a more complex structure in mammals (from 1 to up to 4 chambers, development of an autonomous pacemaker, lining of myocardium by endocardium, development of valves to direct blood flow) (156). These structures are built from multiple cell lineages. The vertebrate heart forms from two embryonic fields derived from the lateral posterior and pharyngeal mesoderm, respectively. The first heart field gives rise to the left ventricle and part of atria, while the highly proliferative second heart field gives rise to the right ventricle, septum, and part of atria and outflow tract (242). Nonmyocardial cell lineages including endothelial cells, smooth muscle cells, and neuronal cells add to the first and second myocardial cell lineages, derived from a common progenitor, to build the whole heart (141).

Cell fate decisions at the origin of all cardiac cell lineages are influenced by both genetic and epigenetic processes. Dysregulation of biological phenomena, such as epigenetically regulated epithelial-to-mesenchymal transition (217), leads to misspecification and disrupted migration of specialized cell types and, in turn, cardiac malformations.

Congenital heart diseases (CHDs) are characterized by various malformations of the heart that can appear from early to late stages of heart development.

CHD is the most frequent birth defect. CHD accounts for 30% of fetal losses and affects 1 out of 100 live births (94) with major malformations in ~1 in 1,000 births. Minor cardiac malformations may be missed at birth while being responsible for cardiac diseases at adulthood and in the aged population (236).

Tetralogy of Fallot (TOF) is the most frequent complex CHD. TOF includes ventricular septal defects, valve defects, aortic displacement, and ventricular hypertrophy. It is a complex disease that results from defects in multiple second heart field cell lineages at the origin of the valves and the ventricular septum (FIGURE 5). Mutations of several genes encoding transcription factors that bind super-enhancers (e.g., NKX2.5, GATAs) or components of signaling pathways (e.g., JAG1, NOTCH, VEGF) have been identified in a small minority of TOF patients (54). Importantly, this is in line with large-scale studies using whole-exome sequencing, which have identified mutations in coding sequences in only a minority of CHD patients. Interestingly, a high proportion of hits in epigenetic modifiers were found (95, 209, 262). Several other diseases with an epigenetic component caused by mutations of structural genes linked to chromatin (i.e., laminopathies), chromatin modi-
fiers (Charge syndrome), or chromatin scaffolders (cohesinopathies) as well as chromosomal anomalies (Down syndrome) include cardiac malformations in their spectrum of pathological traits.

In fact, the genetic and epigenetic bases of the overwhelming majority of CHDs remain largely unknown. This suggests that for most CHDs, a more complex dysregulation of multiple genes and transcriptional pathways occurs, resulting from changes in long-range regulation of transcription. This is probably the result of a disturbance of three-dimensional chromatin architecture and in turn of the epigenetic landscape surrounding regulatory regions of many genes. Thus dynamic changes of both chromatin modifications and structure likely impact cell fate decisions during cardiac development, which in turn plays a role in the occurrence of CHD.

This review summarizes the current knowledge of the impact of epigenetics on cardiac cell fate decisions. It further provides an update on the epigenetic anomalies in the genesis of CHD.

Section II gives an overview of the determination of cardiac cell lineages and how this important process at the origin of the four-chambered mammalian heart is genetically and epigenetically governed.

II. BIOLOGY OF CARDIAC DEVELOPMENT

A. Developmental Origins of Cardiac Cell Lineages

The generation of functional cardiac anatomical structures, a conduction system, and a neuronal network requires a tightly coordinated contribution of different precursor populations throughout development. Although the early embryo exhibits high plasticity and has several redundant mechanisms to ensure its development, aberrant precursor specification and differentiation may lead to congenital defects (7, 105, 106, 156).

A detailed understanding of the early stages of heart development is therefore vital for understanding the etiology of CHDs. Here we describe the developmental origin of cardiac precursors and their early contributions to their respective lineages in the heart.

The decisions on the origins, fates, and lineages of cardiac precursor cells start before gastrulation and continue throughout embryonic and postnatal development. Prior to gastrulation, the embryo consists of an epiblast that gives rise to embryonic structures, a primitive endoderm (or hypoblast) that contributes to extraembryonic visceral endoderm, and trophoblast cells that form the placental structures (231). Analyses in gastrulating embryos revealed a population of cardiac precursors in the lateral posterior epiblast of early streak embryos that ingress during the mid-streak stage through the anterior primitive streak into the space between the ectoderm and definitive endoderm (106, 116, 230, 249) (FIGURE 1, A–C).

Consecutive waves of antero-distal migration ultimately lead to two heart fields becoming located bilaterally of the anterior midline as well as the spatial separation of cardiac precursors of the second heart field (SHF) postero-medially to those of the first heart field (FHF) (144). Retrospective fate mapping, clonal analysis, and lineage tracing studies also suggested that the precursors for the FHF and SHF, including their anterior versus posterior and left versus right descendants, become separated early on and differentially contribute to specific regions of the heart (53, 59, 71, 118, 119, 142).

Shortly after their formation, the bilateral FHF s start to fuse medially to form a crescent-shaped epithelium, known as the cardiac crescent, which then folds ventrally into a trough and starts to close on its dorsal side in anterior and posterior directions, thereby forming the primitive heart tube (158). The heart tube remains connected at the anterior and posterior poles to the dorsally located pharyngeal mesoderm where the SHF emerges (2) (FIGURE 1, D–F).

While the cardiomyocytes from the heart tube expand by proliferation to form the primitive left ventricle as well as part of the atria and right ventricle (50, 143), cells from the SHF migrate via the anterior and posterior poles into the heart and contribute to the outflow tract, right ventricle, atria, and inflow tract (235) (FIGURE 1, G–J). While the FHF primarily gives rise to cardiomyocytes (124, 216), SHF precursors are considered to be multipotent, giving rise to cardiomyocytes, smooth muscle cells, endothelial cells of the heart, outflow tract and inflow tract, as well as cardiomyocytes of the conduction system (34, 119, 159, 174, 245).

The early heart tube is lined on the inside by the endocardium, which develops simultaneously with the myocardium of the primitive heart and, after looping, becomes covered on the outside by epicardium. The embryonic origin of endocardial precursors remains controversial (87, 183). Lineage marking studies suggested that endocardial and myocardial precursors have a common origin (34, 103, 150). In contrast, clonal analyses showed that endocardial and myocardial precursors are uniquely specified just before or during gastrulation (46, 248). Despite the different views on its origin, once formed, the endocardium is important for conduction system development via paracrine signaling towards the underlying myocardium (189) and direct cellular contributions as well as signaling to coronary vessels and the developing valves in the outflow tract and atrioventricular canal (41, 254).
Between embryonic day (E) 6.5 and E7.5 (A–C), cells from the primitive streak (PS) ingress into an antero-distal direction in consecutive waves, giving rise to cardiac precursors of the first heart field (FHF, red) and anterior and posterior second heart fields (orange-green aSHF and green-blue pSHF, respectively). The bilateral heart fields fuse at the level of the anterior midline during the cardiac crescent stage (E7.5; D). Cells from the FHF give rise to the primitive ventricle (PV, red; E). The aSHF contributes to arterial structures like the outflow tract (OFT, orange-green; E and F) and the bulbus cordis (BC, orange; F). The pSHF contributes to formation of venous structures such as the common atrium (CA, green-blue; G) and right and left sinus horns (RSH, LSH; E). After looping, these primitive structures further develop and become septated into left ventricle (LV, red) and right ventricle (orange), aorta (Ao) and pulmonary artery (PA, orange-green), right and left atria (RA, LA, green-blue), superior vena cava (SVC, blue), and coronary sinus (not shown) (G–I). The sinoatrial node (SAN, not shown) derives from the pSHF and ultimately resides at the border of the SVC and RA, whereas the dorsally located atrioventricular node (AVN, not shown) is thought to be derived from the FHF. A, anterior; P, posterior; Pr, proximal; D, distal; R, right; L, left. A–C are lateral views, and D–I are ventral views.
The epicardium derives from an extracardiac structure called the proepicardium, which develops in the pericardial space between the cardiac inflow tract and septum transversum (reviewed in Refs. 195, 214). Proepicardial and SHF myocardial precursors derive from a common population, and their respective cell fates are affected by signaling in the venous pole of the heart (114, 238, 268). Cells from the highly heterogeneous proepicardium travel via the pericardial space to the developing heart and form the epicardium. They subsequently undergo epithelial-to-mesenchymal transition (EMT) and invade the myocardium giving rise to coronary smooth muscle cells, pericytes, fibroblasts, endothelial cells (104, 146), and valvular cells (250).

Shortly after myocardial precursors have started migrating in the gastrulating embryo, ectodermal neural crest cells delaminate from the neuroepithelium and contribute to, among others, craniofacial structures and the nervous system. A subset of cells from the neural crest, called cardiac neural crest cells, travels from the embryonic posterior hindbrain via the pharyngeal arches to the anterior heart and contributes to mesenchymal and smooth muscle cells of the outflow tract and coronary arteries and neurons of the cardiac ganglia and nervous system (12, 107, 226).

After formation of primitive cardiac structures and cell layers by the aforementioned precursor populations, the arterial and venous blood flows are separated from each other and valves are formed to ensure unidirectional flow (reviewed in detail in Refs. 8, 9). Cardiac neural crest cells invading into the endocardium-derived cushions contribute to remodeling of the common outflow tract into aorta and pulmonary artery. Septation of atrial chambers is established by the primary atrial septum as well as the endocardium-derived mesenchymal cap and atrioventricular cushions and SHF-derived mesenchymal cells that protrude from the dorsal mesocardial wall into the atrial lumen. Together with cardiomyocytes from the interventricular septum, the atrioventricular cushion-derived mesenchymal cells close off the connection between the ventricles. The primitive valves in the atrioventricular region are initially derived from endocardium-derived mesenchymal cells, although an epicardial contribution to the maturing parietal valves was reported as well (250).

B. Transcriptional Regulation and Genetic Networks

Heart development is regulated by intricate networks of transcription factors (TFs) (30). Mice in which some of these key TFs have been targeted by transgenic approaches have provided insight into their function and identified the precursor populations that rely on these genes. The earliest mesodermal precursors, that ultimately give rise to the FHF and SHFs, express the transcription factor Eomesodermin (Eomes), which is broadly expressed in the pre-streak posterior epiblast and balances the development of cardiac precursors versus definitive endoderm (48). Eomes, together with Oct4 and Wnts, activates the transcription factor Mesp1 in the earliest cardiac progenitors (FIGURE 2), which is thought to regulate the EMT that is required for delamination of the initially sheetlike population of ingressing cells (1, 48, 122, 130, 133, 175, 263). Analysis of Mesp1lacZ and Mesp1Cre mouse models showed that Mesp1−/− precursors migrate away from the primitive streak and contribute to cardiac precursors of the heart tube, endoderm, and epicardium, but not to cardiac neural crest derivatives (118, 193). Interestingly, labeling of Mesp1lacZ precursors in gastrulating embryos suggested that precursors contributing to the FHF and SHF become specified at early to mid versus late primitive streak stages, respectively (56, 126), confirming earlier findings on the gradual spatiotemporal separation of FHF and SHF precursors (59, 71, 119, 141). Disturbance of Mesp1 results in severe defects and embryonic lethality (108, 193).

In embryonic stem cell-derived mesodermal cells, Mesp1 (indirectly) regulates cardiac transcription factors

![FIGURE 2. Transcriptional pathways and morphogens governing myocardial cell lineage determination. Morphogens are in brown, and transcription factors are in black. Blue arrows indicate activation, and red arrows indicate inhibition.](https://example.com/figure2.png)
Mef2c, Nkx2–5, and Isl1 (23). This currently controversial view (215) suggests that Mesp1 may act upstream of a hierarchy of cardiac genetic regulation in mesendoderm. Mef2c and Nkx2–5 are first expressed in the fused first and second heart fields, with Nkx2–5 being additionally expressed in endocardium and pharyngeal endoderm, but not in the precursors that give rise to the sinus venosus myocardium and sinoatrial node (44, 62, 131, 153–155, 182, 267).

Mef2c and Nkx2–5 can both act as either transcriptional activators or repressors and interact with numerous other transcription factors to regulate cardiac precursor specification, proliferation, and differentiation (73, 89, 93, 182, 243). Mutations in Mef2c or Nkx2–5 lead to changes in these interactions and, consequently, cardiac structural and/or functional defects (21, 27, 152, 198).

Nkx2–5 directly interacts with the transcription factor Tbx5 (32, 93), which has a very distinct spatiotemporal expression pattern in the FHF and SHF (31). Tbx5 mutations lead to several types of congenital heart defects in Holt-Oram patients and mouse models (16, 32, 121, 162, 256). Tbx5 and Nkx2–5 interaction is important for the development of the sinus node as well as ventricular conduction system (162, 163, 184). Additionally, Tbx5 acts upstream and/or parallel to pulmonary endoderm–derived hedgehog signaling during atrial septal formation (256) consistent with phenotypes seen in patients.

Both Nkx2–5 and Tbx5 interact with Gata4 and mutations in either of these TFs result in atrial septal defects (61, 72, 151). Additionally, Nkx2–5 regulates Gata6 activity (151) and is itself regulated by Gata4 (29) (FIGURE 2), suggesting a strong interdependence of these transcription factors. Gata4 and Gata6 are coexpressed in cardiac precursors at primitive streak stages, with Gata4 being additionally expressed in visceral endoderm, and both are later expressed in primitive myocardium and endocardium (91, 161). At midgestational stages, Tbx5 is coexpressed and interacts with Gata4 and Gata6 in the atrial septum and atrioventricular cushion. Atrial and atrioventricular defects are more severe in mice that are compound heterozygous for Tbx5 as well as Gata4 or Gata6 (139).

In addition to Tbx5, several other Tbx transcription factors are expressed during early development and involved in patterning of cardiac precursors (37, 112, 113, 208). Precursor cell proliferation and differentiation are regulated by Tbx1 in the SHF; Tbx2 and Tbx3 in the atrioventricular canal and conduction system; Tbx18 in sinus venosus myocardium, sinus node, and epicardium; and Tbx20 in myocardium and endocardium (79). Tbx20 represses Tbx2 in the chambers (FIGURE 2) to restrict its expression domain to the atrioventricular canal (212). Tbx1 regulates the expression pattern in the FHF and SHF (31).

Nkx2–5 is additionally expressed in the proepicardium and regulates epicardial EMT and formation of coronary smooth muscle cells, pericytes, and fibroblasts (255). Finally, Tbx20 is first expressed in myocardial and endocardial precursors of FHF and SHF and was reported to indirectly inhibit BMP2 activation of Tbx2 via sequestering Smad1/5 or to interact with Isl1 and Gata4 to activate expression of Mef2c and Nkx2–5 (33, 112, 211, 212, 220, 228).

Isl1 is first expressed in the bilateral SHFs and underlying endoderm. It remains expressed during further SHF development and in SHF cells migrating into the anterior and posterior poles of the heart (34, 182, 261). Isl1 becomes downregulated upon differentiation, but remains expressed in cardiac progenitors and conduction system cells of postnatal hearts (115, 125). Isl1 expression was also found in extracardiac lineages, including Wnt1 expressing neural crest and Tbx18+/Wt1+ pro-epicardium (63, 138, 182, 224, 268). This broad cellular requirement for Isl1 and the observations that it takes part in genetic feedback loops with Mef2c and Nkx2–5 (58, 60, 239) further highlight the complex genetic regulation of cardiac development.

Mef2c, Nkx2–5, and Isl1 all regulate expression of Hcn4 (154, 239, 240), which is involved in the activation of pacemaker cells in the SHF-derived sinus node and FHF-derived atrioventricular node of the conduction system (140). Recently, studies in murine primitive streak stage embryos revealed that Hcn4+ precursors also contribute to descendants in the FHF-derived left ventricle. Hcn4 expression is downregulated in the left ventricle during cardiac maturation and becomes confined to the sinus node and atrioventricular node of the conduction system (124, 216). Although Hcn4 expression is necessary for a functional conduction system, the primitive left ventricle still develops normally in the absence of HCN4 (221), suggesting that it is not required for specification or differentiation of FHF precursors.
III. EPIGENETIC REGULATION OF GENE TRANSCRIPTION

Throughout development and during their lives, organisms must tightly regulate the expression of thousands of genes in millions of cells. Over 20,000 genes are encoded in the DNA sequence of the human genome. Although all (somatic) cells contain this same genetic material, highly regulated gene activity enables the emergence of multiple cell types and functions. Gene activity largely depends on transcriptional regulation, which is in turn orchestrated by epigenetic mechanisms. These heritable modifications to DNA, DNA-associated proteins and chromatin structure, preserved during cell division, regulate accessibility to DNA. This has led to the concept of chromatin states, with open euchromatin associated with gene activity and closed heterochromatin where gene activity is repressed. Importantly, epigenetic mechanisms (i.e., DNA methylation, histone modifications, higher-order chromatin structure) have become the focus of large efforts to better understand the etiology of human diseases and may provide a novel therapeutic window.

Section III gives an overview of epigenetic factors, from local chemical DNA modifications to three-dimensional architectural changes that regulate chromosome structure and accessibility for transcriptional regulation. In particular, we focus on the DNA looping processes, occurring within insulated neighborhoods, mediated by the cohesin and mediator complexes. Section IV discusses these epigenetic factors in the context of cardiac development and diseases.

A. Modifications to DNA and Histones

1. Chemical modifications to DNA

DNA methylation refers to the covalent addition of a methyl group from the methyl donor S-adenosylmethionine (SAM) to a cytosine base within the DNA by DNA methyltransferases (DNMTs). In mammals, methylation is restricted to CpG dinucleotides, which are largely depleted from the genome except at short genomic regions called CpG islands, which commonly represent promoters. Methylation of CpG-rich gene promoters is associated with repressed chromatin and reduced gene transcription.

DNA methylation can interfere with transcription factor binding, yet repression seems to occur largely indirectly, via recruitment of methyl-CpG binding domain (MBD) proteins that induce chromatin changes.

DNA methylation patterns are erased during gametogenesis and in early development, with waves of methylation occurring during post-implantation. Methylation is catalyzed by DNMTs, including Dnmt1, Dnmt3A, and Dnmt3B. De novo methylation is carried out by both Dnmt3A and Dnmt3B that are essential for embryonic development. Dnmt1 is considered to be the main enzyme responsible for the maintenance of methylation, i.e., the transfer of methylation patterns to daughter cells, and DNMT1 disruption results in loss of cell proliferation and cell death. Conversely, demethylation is achieved by the activity of ten-eleven-translocases (TET) enzymes. TET1 has been shown to drive DNA demethylation by converting 5-methylcytosine to the intermediate 5-hydroxymethylcytosine.

The DNA methylation status of ~80% of the CpGs in the human genome is stable. It does not change significantly between different cell types or developmental stages, thus is not expected to be causative of diseases. However, aberrant methylation patterns have been associated with disease contexts such as cancer, notably including hypermethylation of promoters of tumor-suppressor genes. Early evidence for the importance of methylation in determining gene expression, and an early example of a drug targeting an epigenetic process, came from a study showing that treatment of cells with 5-azacytidine, that inhibits methylation, leads to the emergence of novel phenotypes.

2. Posttranslational modifications to histones

Encoding genes are tightly packed within a scaffold of chromatin that not only ensures a protective environment but also regulates their expression in a time- and cell lineage-specific manner. Nucleosomes are the scaffold repetitive units that are wrapped by 147 bp of DNA. They are composed of octameres of histones, H2A, H2B, H3, and H4 and are organized into higher-order structures to further condense the DNA. Modifications of the histones (i.e., methylation, acetylation, phosphorylation, and sumoylation) confer a gene regulatory property to the nucleosome. Histone methyltransferases, demethylases, acetyltransferases, and ATP-remodeling factors together compose the chromatin remodeling complexes.

Histone methyltransferases, demethylases, acetylases, and deacetylases are multi-domain proteins, which likely explains their multifunctional requirements. These include their targeting to the nucleosome and specific histones through a reader domain, their DNA binding and enzymatic activity, and their association to other proteins in transcriptional factories.

Mutations in many of these enzymes are responsible for cardiac congenital diseases. Histone methyltransferases (HMTs) able to fine-tune the methylation state of histones include the MLL family proteins with a SET domain. These include the targeting to the nucleosome and specific histones through a reader domain, their DNA binding and enzymatic activity, and their association to other proteins in transcriptional factories.
Histone acetyltransferases provide an important epigenetic regulation that lead to transcriptionally active, noncompact chromatin. Conversely, removal of transcriptionally permissive modifications, such as H3K4me3 by KDM5c and then LSD1 (H3K4me2 and H3K4me1), requires the Co-REST complex, including histone deacytelases 1/2 (HDAC1/2) and the PHD fi1/2 protein BHC80 (260). The protein complex leads to the formation of more repressive chromatin states. Interestingly, these studies have revealed that most of the abundantly methylated lysine residues in histones have a corresponding demethylase enzyme.

Histone acetyltransferases provide an important epigenetic regulation that lead to transcriptionally active, noncompact chromatin. CBP (Creb binding protein) and p300 are the most characterized HATs and are essential for heart development (206).

Histones are deacetylated by deacetylases (HDACs). These include both Zn²⁺-dependent histone deacytelases (83) (HDAC1–11) and NAD⁺-dependent sirtuin deacytelases (SIRT1–7). Sirtuins are localized within the nucleus, cytoplasm, and mitochondria of the cell, whereas Zn²⁺-dependent deacytelases are absent from mitochondria.

HDACs have many roles in cardiac development and diseases (83). HDACs 1, 2, 3, and 8 knockout feature gastrulation, cardiac, and craniofacial defects. Cardiac conditional deletion of HDAC1 and 2 together has revealed that a single wild-type allele of either gene is sufficient to support cardiac development. However, biallelic deletion of both HDAC1 and 2 leads to cardiac arrhythmias, dilated cardiomyopathy, and in turn death at birth.

Among the first discovered demethylases, KDM1A/LSD1 removes methylation from histone H3 on lysine 4 (H3K4) when di- or monomethylated using its amine oxidase domain (205). KDM2A/JHDM1A/FBXL11 erase methyl marks from H3K36 via their JmjC domain (234). Since these enzymes were first uncovered, an extended family of related demethylase enzymes has been further characterized (22, 205).

Demethylases, by removing methyl groups from histones, establish new chromatin environments at gene regulatory regions. Removal of repressive marks, such as H3K27me2/3, by the demethylase JMJD3 leads to transcriptionally permissive chromatin. Conversely, removal of transcriptionally permissive modifications, such as H3K4me3 by KDM5c and then LSD1 (H3K4me2 and H3K4me1), requires the Co-REST complex, including histone deacytelases 1/2 (HDAC1/2) and the PHD fi1/2 protein BHC80 (260). The protein complex leads to the formation of more repressive chromatin states. Interestingly, these studies have revealed that most of the abundantly methylated lysine residues in histones have a corresponding demethylase enzyme.

Histone acetyltransferases provide an important epigenetic regulation that lead to transcriptionally active, noncompact chromatin. CBP (Creb binding protein) and p300 are the most characterized HATs and are essential for heart development (206).

Histones are deacetylated by deacetylases (HDACs). These include both Zn²⁺-dependent histone deacytelases (83) (HDAC1–11) and NAD⁺-dependent sirtuin deacytelases (SIRT1–7). Sirtuins are localized within the nucleus, cytoplasm, and mitochondria of the cell, whereas Zn²⁺-dependent deacytelases are absent from mitochondria.

HDACs have many roles in cardiac development and diseases (83). HDACs 1, 2, 3, and 8 knockout feature gastrulation, cardiac, and craniofacial defects. Cardiac conditional deletion of HDAC1 and 2 together has revealed that a single wild-type allele of either gene is sufficient to support cardiac development. However, biallelic deletion of both HDAC1 and 2 leads to cardiac arrhythmias, dilated cardiomyopathy, and in turn death at birth.

Double knockout of both HDAC5 and HDAC9 features lethal ventricular septal defects (VSD) and thin-walled myocardium (83).

Recent studies link histone acetylation and chromatin modifications with metabolism. Hence, histone acetylation may respond to changes in cell metabolism and be a sensor for nutrition-based epigenetic regulation of gene transcription (43, 210). It is important to note that enzymes regulating acetylation and methylation are not specific to histones or DNA, and hence are thought to regulate gene expression by acting on other proteins such as transcription factors and DNA repair proteins. For example, HDACs can regulate transcription without catalyzing deacetylation. Notably, the class IIa HDACs (HDACs 4 and 5) are involved in the formation of repressor complexes that impede the activity of the pro-hypertrophic transcription factor myocyte enhancer factor-2 in cardiac myocytes (137, 266).

B. Genomic Architecture

Nucleosome organization is the first level of chromatin organization, enabling DNA to be packaged into chromatin fibers. Initially, electron microscopy showed that nucleosomes were organized leading to the “30 nm chromatin fibers” (233). This has been challenged, and super resolution nanoscopy of nucleosomes in individual nuclei suggests they are assembled in “clutches,” distinct nucleosome groups of variable sizes found dispersed along the chromatin fiber (190). How DNA is folded within the nucleus is an area of considerable debate, and the implications are immense as the mechanisms regulating genome organization clearly drive gene expression in development and pathological contexts.

A number of early studies revealed the presence of cis-acting DNA sequences that control gene expression, notably of the beta-globin gene (80, 253). It has since been established that cell type specific transcriptional programs are controlled by enhancers, DNA elements that recruit transcriptional regulators such as transcription factors to target genes (98). In every cell, thousands of enhancers are physically associated with expressed genes. Cooperation between cell specific transcription factors at enhancers may confer another level of specificity to enhancer activity. Enhancers interact with distant genes, usually on the same chromosome, via chromatin folding and “loop” formation. Enhancers may be subject to mutations and polymorphisms that are at the origin of developmental diseases (244).

1. Chromosome structure

Very early evidence of structural organization of chromatin at a higher level came from the observation of “chromosome territories,” referring to individual interphase chromosomes located to confined spaces within the nucleus.
rather than being randomly dispersed (49). Such organization suggested a predominance of intrachromosomal versus interchromosomal interactions. The advances of 3C technologies (52), leading to Hi-C (127), confirmed this speculation, notably leading to the current model whereby chromosomes contain evolutionary conserved regions with relatively high levels of internal interactions known as TADs (topological associated domains) (57, 203). TADs (FIGURE 3) are identifiable as regions with epigenetic traits, such as active chromatin and high levels of internal loop formation and limited by boundaries, formed by architectural proteins such as CTCF (168). Microscopy approaches, notably FISH, as well as chromatin immunoprecipitation assays combined with next generation sequencing have provided direct evidence for these properties at the level of individual cells.

Although there is clearly a consensus regarding the presence of chromosomal domains (TADs), characteristics such as size vary depending on the studies, likely due to the presence of domains (sub-TADs) within TADs and the increasing resolution of Hi-C (24). At a higher level, compartments A and B refer to relatively active and inactive regions, respectively, within which TADs tend to interact (127). Interestingly, it is thought that TADs are mostly conserved among cell types, but compartment organization varies in a cell type-specific manner (56).

2. The architectural proteins

The so-called architectural proteins are required for mediating DNA folding within the nucleus and regulate the activity of enhancers, promoters, and insulators (FIGURE 2).

**FIGURE 3.** Epigenetic components [DNA methylation, histone modifications, topological associated domains (TADs), and lamin-associated domains (LADs)] playing a role in cardiac development. PRC1/2, polycombs; NPC, nuclear pore; Me, methyl group.
A) CTCF. Insulator complexes are regulatory elements that block enhancer-promoter interaction when situated between that enhancer and promoter. The transcription factor CTCF binds to insulators (20), but only a small proportion of all CTCF binding sites are actually at insulators. Furthermore, CTCF is found at boundary elements that separate TADs. These are identifiable as regions with strong loops, and hence are also known as “loop domains” (186). CTCF is thus required and essential from early to late stages of heart formation (76).

B) MEDIATOR. The mediator complex comprises 26 subunits in mammals and is a dynamic structure, with subunits being lost or exchanged during events that are currently not well-defined. As an example, mediator associates with the CDK8 module including CCN, MED12, and MED13 (3, 6). The mediator complex binds super-enhancers and associates with pioneer factors (136) pointing to a function of the complex in cell differentiation and organism development. Mediator (MED12) also links epigenetics and mitochondrial genome and metabolism (204).

Importantly, the mediator complex further acts as a link between transcription factors and RNA polymerase II and interacts with cohesin at actively transcribed genes in the presence of NIPBL (106).

3. The cohesin complex

Cohesin is a multi-protein complex formed by four subunits, including the maintenance of chromosome proteins family SMC1/3, a kleisin protein RAD21, and a HEAT domain protein SA/STAG. Several other proteins are associated with this complex. These include SGOL1 and protein phosphatase 2 proteins, which protect the cohesion complex from unloading, and PDS5 and WAPL, the unloading complex. Finally, NIPBL and its MAU partner form a heterodimer that serves as a DNA loader of the cohesin complex. The cohesin complex forms a ring around chromosomes, and its first uncovered function was the cohesion of sister chromatids. More recently, cohesin has been shown to be involved in the regulation of gene transcription and has a key function in maintaining the memory of transcription factors binding sites during S phase of cell mitosis (258) (FIGURE 4).

Kagey et al. (102) showed how mediator recruits cohesin to promote gene expression, notably at “super-enhancers,” involving multiple enhancers bound by master regulatory TFs that play key roles in determining cell fate decisions and maintaining cell identity (102). TFs can be found in clusters involving tens of TFs, and cohesin is almost systematically present at clusters. TFs mostly dissociate from chromatin during the cell cycle, and cohesin has been shown to facilitate the reestablishment of TF binding, acting as a “cellular memory” (258). In mammalian cells, cohesins have been found to be enriched at conserved noncoding sites and sites of CTCF binding. CTCF knockdown prevents cohesin positioning (169), and CTCF binding is abolished by DNA methylation (86). Somewhat surprisingly, NIPBL was not found to be associated with cohesin at CTCF sites, suggesting that CTCF recruits cohesin at insulators, whereas NIPBL is required at sites of active transcription involving mediator cohesin complexes that contribute to loop formation (102).

In agreement with this key role of the cohesin complex in regulating gene expression, it has been shown that cohesin and its partners are required for normal gene expression. Rollins et al. (192) showed that the cohesin loading partner Nipped-B/delangin was required for the activation of the cut gene in Drosophila. Furthermore, RNA knockdown experiments performed in vitro revealed that a particular chromatin conformational change underlying early mesoderm formation was highly sensitive to levels of Nipbl targeted by the pioneer factor Oct4 (1).

C. Lamin-Associated Domains

The inner nuclear membrane of eukaryotic cells is lined by the nuclear lamina (NL), a fibrous layer consisting primarily of type V intermediate filament proteins named lamins. Chromatin associated with the NL has been found to be repressed. Notably, using fluorescence in situ hybridization, Kosak et al. (111) showed that actively and inactively transcribed immunoglobulins were centrally and peripherally positioned, respectively, in the nuclei of hematopoietic cells. Using DamID technology, involving the generation of a Dam-Lam fusion protein that labels chromatin associated with Lamin, Pickersgill et al. (177) showed that lamin-associated genes were silenced. Hence, lamin-associated...
IV. EPIGENETIC REGULATION OF CARDIAC DEVELOPMENT

At a first level of regulation, DNA methylation status of cardiac genes have also been linked to congenital heart diseases (202). The Sperling group (81) reported that differential methylation of distinct CpG islands located in the promoter significantly reduces expression of SCO2, a cytochrome oxidase. Hypermethylation of this region was found in TOF and VSD patients. Interestingly, these CpG may harbor binding sites of TFs playing a role in early cardiac development. Other dysregulations in DNA methylations were found linked to gene expression or repression in heart samples of TOF and VSD patients (81).

Another example of a significantly downregulated gene with hypermethylation of its promoter is ECE2, an endothelin substrate. This has also been associated with impairment in cardiac development including VSD (259). How DNA methylation status changes in the course of the diseases is still poorly known, although this is an emerging and active field of research. Whether DNA mis-methylation is part of a causative process of CHDs is still too early to be established.

DNA methylomes from purified cardiomyocytes of neonatal, adult healthy, and adult failing hearts have revealed a dynamics of DNA methylation under specific developmental or pathological conditions. Cardiomyocyte development was accompanied by a change in methylation status of DNA. DNA methylome consisted of a demethylation wave running all over embryonic life (at least from E14.5) through cardiac gene bodies, including genes encoding sarcomeric proteins, which leads to de novo DNA methylation after birth. Deposition of polycomb marks in embryonic myocytes can still repress transiently expressed demethylated genes. Altogether these processes shape the epigenome during development and at prenatal stage before cardiomyocyte maturation (74). Using human fetal and neonatal cardiomyocytes, the authors further revealed a highly dynamic interaction between DNA methylation and histone marks in the course of human heart development (75).

To further get insight into the process of DNA methylation, Nothjunde et al. (166) studied the impact of three-dimensional chromatin structure on DNA demethylation. To such an aim, they investigated spatially segregated A (mono-TAD) and B (multi-TAD) compartments that are active (enriched in H3K27ac, H3K4me1/me3, H3K9me1, and H3K27me3 histone marks) and inactive heterochromatin (enriched in H3K9me3 mark), respectively. They found that the higher order chromatin configuration guides the cell specific DNA methylation (166).

Hydroxymethylation [hydroxymethylcytosine (5-hmC)-5-mC’s] of DNA also recently emerged as an important regulator of development and diseases (123). In a cardiac context, Greco et al. (78) mapped the hydroxymethylome of embryonic, neonatal, and adult cardiomyocytes. They found that DNA hydroxymethylation was present on highly expressed genes as well as on distal regulatory regions of cardiac genes and was correlated with gene transcription. However, in the course of cardiac development, 5-hmC was lost mostly accumulated on gene bodies. Enrichment of gene promoters in 5-hmC was associated with mild repression of gene expression in embryonic and neonatal cardiomyocytes, and no effect on adult (78). In vivo studies are still missing to conclude about the involvement of DNA hydroxymethylation in CHDs.

At a more complex level of regulation, ATP-remodeling factors have been involved in cardiac congenital diseases. There are four different families of SWI-like ATP-dependent chromatin-remodeling complexes: 1) the SWI/SNF (switching defective/sucrose nonfermenting) including the main ATPase subunit of the complex Brg1; 2) ISWI (imitation switch); 3) CHD chromodomain, helicase; and 4) INO80 (inositol requiring 80) complexes. These complexes play a crucial role in cardiac development from early stages.

Brg1/Brm associated factor (BAF), a chromatin remodeling factor, allows for a proper activation of enhancers of mesodermal genes in differentiating embryonic stem cells. It co-localizes with the epigenetic mark acetylated H3K27 at gene enhancers. Interestingly, Brg1 co-represses nonmesodermal genes by recruiting the polycombs to these loci and by depositing H3K27me3 (5).

Brg1 is also a seminal chromatin remodeling factor to ensure proper cardiac formation. Early deletion of Brg1 impaired cardiomyocyte proliferation by decreasing BMP10 and in turn inducing ectopic expression of p57kip2, a cyclin-dependent kinase inhibitor usually under the transcriptional control of BMP10 (85). The BAF factor interacts with HDACs and PARPs [poly(ADP-ribose) polymerase] to repress α-myosin heavy chain (MHC), the adult form of MHC in mice, and to activate the embryonic isoform β-MHC. This maintains the embryonic phenotype of the heavy chain of myosin.

Using both zebrafish and mouse models of Brg1 haploinsufficiency, Takeuchi et al. (228) investigated the effect of cooperation of Brg1 with cardiac TFs. They found that Brg1 functions in a dose-dependent manner. Haploinsufficient hearts featured trabeculation defects and low proliferation rates of cardiomyocytes. Gata4, Nkx2.5, and Tbx5 activities all depended at least in part on Baf60c, a key member of the BAF complex (92, 126). Embryos haploinsufficient for both Brg1 and Nkx2.5 or Tbx5 displayed even more profound heart defects, thus pointing to a fine balance
between the level of expression of Brg1 and those of cardiac transcription factors. Brg1 is also expressed in endocardium and regulates in a cell autonomous and cell non-autonomous manner the composition of the cardiac jelly that forms the cushions at the origin of the valves. This chromatin remodeler modulates secretion of the metalloproteinase Adams1 (218). Brg1 was most recently reported to interact with thymosin β4 and to be recruited by C/EBPβ at the Wt1 locus. This complex regulates Wt1 transcription and in turn the determination of the epicardial lineage (241) and sub-lineages of epicardial-derived cells.

A role of other BAF subunits has been uncovered in the course of cardiac development. Baf60c activity is necessary in the mouse embryo to establish right-left asymmetry (227). It is then further required for the determination of cardiac progenitors. Baf60c may link transcription factors with the BAF complex ATPase Brg1 (126).

Baf180 is another key BAF subunit required for normal heart formation. Mice deficient in Baf180 die in utero by E15.5, likely of heart failure due to ventricular septal defects and hypoplastic ventricles. The cardiac phenotype is close to the one observed in retinoic acid receptor alpha (RXRα) knockout embryos, and Baf180 has been reported to be essential for expression of retinoic acid target genes (247).

Epigenetic regulation of cardiac development can additionally occur via histone modifications. Tbx genes have been shown to associate with demethylases JMD3 and UTX JMD3. UTX mediates a functional interaction between the lineage-defining T-box transcription factor family and a Brg1-containing SWI/SNF remodeling complex (147, 148). Although not investigated in cardiac cells yet, the role of Tbx5 in heart development is mandatory and their interaction with JMD3 and UTX is likely to play a function. UTX was indeed reported to play a key role in cardiac cell differentiation of ES cells as well as in heart formation. UTX-deficient ES cells failed to differentiate in cardiomyocytes. UTX null mice featured a linear tube heart indicating a failure in looping at E8.0. UTX was normally recruited to cardiac gene enhancers in wild-type embryonic hearts and demethylated H3K27 (117).

Cattaneo et al. (35) screened 85 genes encoding the epigenetic-related proteins in mouse cardiomyocytes. They found that DOT11 (H3 methyltransferase disruptor of telomeric silencing 1-like) was the most expressed. Using the in vitro ES cell model, they showed that DOT11 shaped dimethylation on H3 lysine 79 (H3K79). Knockdown of Dot11 in ES cells impacted expression of H3K79me2-enriched cardiac genes (35).

KMT2D and KDM6A mutations are responsible for Kabuki syndrome, a developmental syndrome including cardiac malformation. Deletion of KMTD2 in zebrafish recapitulated the disease (237).

Polycombs are part of a repressive complex composed of two units: PRC1, which ubiquitylates H2AK119, which itself is mediated by Ring1 and 2 ubiquitin ligases and coregulated by JARID2 featuring a ubiquitin interaction motif (47), and PRC2 that methylates H3K27, which is mediated by Ezh1 and Ezh2.

The role of polycombs in mesodermal differentiation of ES cells was delineated by Morey et al. (160). Polycomb complexes (PRC1 and 2) are major regulators of transcription as they repress this process. Mel18, which regulates PRC1, functions as a polycomb at early stages of cardiac mesodermal lineage determination. It allows for expression of many mesodermal and cardiac genes including Brachyury, Mesp1, Flk1, GATA4/6, Tbx5, Nkx2.5, and Tbx20 in ES cells that are differentiating into cardiomyocytes. Mel18 functions as a co-regulator of PRC1 which acts either as a repressor or as an activator of gene transcription in mesoderm and early cardiac progenitors, respectively (160).

Polycombs were also reported as crucial regulators of cardiac development in vivo. Conditional knockout of Ezh2 (a component of PRC2) using a Nkx2.5 Cre mouse leads to major cardiac malformations including ventricular and atrial septal defects as well as chamber trabeculation defects (40). Defects in endocardial cushion formation were also observed (40). Interestingly, specific deletion of Ezh2 in cardiomyocytes using cTnT Cre mouse led to a mild embryonic phenotype, mostly a decrease in cardiac cell proliferation. However, Ezh2 haploinsufficient mice die after birth. This phenotype was more severe in an Ezh1 knockout background. Mice with global knockout of Ezh1 combined to deletion of Ezh2 in cardiomyocytes featured VSDs and abnormal chamber trabeculation and heart hypoplasia (4).

Histone modifying enzymes such as HDACs, HATs, and methyltransferases also play major roles in the course of cardiac development. The laboratory of Eric Olson (83) has made a major contribution in delineating the role of HDACs in cardiac development and growth. This has been previously reviewed (83), and we will not further develop this issue. It is however important to highlight that among HDACs, HDAC5 and 9 have an impact on heart formation. Deletion of either HDAC5 or 9 results in VSDs (36).

HDAC8 mutations are responsible for a cohesinopathy. HDAC8 deacetylates SMC3, a component of the cohesin complex. Mutation of HDAC8 impairs the acetylation process which prevents the cohesin complex from being released from chromatin in both the prophase and anaphase (51). Among the histone deacetylases, Jumonji, a member of the Jumonji family of histone demethylases, is required to
build the heart. Its knockout in mouse leads to double outlet right ventricle (DORV) and hypertrabeculation (229).

Germline deletion of Smyd1, which encodes HMTs, causes ventricular hypoplasia (170). A point mutation of p300, a member of the HATs, leads to heart defects such as ASD and VSD (206). Histone methyltransferases are also partners in the cardiogenic transcriptional program (10). KMTD2, which transfers methyl groups to H3K4, is a major regulator of gene transcription through activation of enhancers and promoters at loci binding H3K4me1 and H3K4me2, respectively. KMTD2 mutations lead to haploinsufficiency and a cardiac congenital syndrome, the Kabuki syndrome. Deletion of KMTD2 in the Mesp1 lineage in mice results in delayed heart development and is embryonic lethal after E10.5. Deletion in cardiomyocytes with TntCre leads to embryonic death by E14.5. Specific deletion in the anterior heart field using Mef2c Cre gives a severe phenotype with no live embryos at E13.5. Thus KMTD2 is mandatory for proper cardiac development (10).

Histone H3 trimethylation at lysine 36 (H3K36me3) is a key gene transcription regulator epigenetic mark. Wolf-Hirschhorn syndrome candidate 1 (Whsc1), the histone methyltransferase that methylates H3K36, is mandatory to form a healthy heart. Its knockout in mice leads to atrial septal defects and VSD (165).

V. (EPI)GENETIC DISEASES

Genetics often cannot explain the full phenotype of so-called genetic diseases or the patient-to-patient variability in the expression of the phenotype. Epigenetic features likely contribute either as a trigger or to the development of some genetic diseases. We will discuss this issue in several cardiac congenital diseases.

A. Down Syndrome

Down syndrome (i.e., trisomy 21) is among the most frequent developmental diseases with a frequency of 1 in 700 live births. Although intellectual disabilities are the main feature, many children (~40%) also suffer from cardiac congenital diseases (11). Atrioventricular defects are the most frequent form of CHD. Ventricular septal and atrial septal defects are also found as well as TOF (68) (Figure 5). Significant progress in describing the molecular mechanisms of the disease has been accomplished since the sequencing of HSA21 16 yr ago (88). Trisomy 21 is considered as a disorder of gene expression. Surprisingly, not only genes like HSA21 are dysregulated but also genes on other chromosomes. This suggests that interchromosomal interactions may play a role in the disorder. Dysregulated genes are organized in domains called gene expression dysregulation domains (GEDD). Interestingly, GEDDs correlate with LADs. LADs are however not affected in cells from trisomy 21 patients. The H3K4me3 epigenetic mark that decorates the GEDDs is affected in fibroblasts of trisomy 21 patients. This indicates that the syndrome is in part a chromatin disorder. Overexpression of genes harbored on HSA21 may be at the origin of chromatin modifications. For example, the HMG1 gene (high mobility group nucleosome binding domain 1) encoding a factor that modifies histones and influences chromatin structure (179) is dysregulated in Down syndrome patient cells. The disease was recapitulated using a HUES cell line derived from a sibling following PGD (25). The authors found a reduced number of Isl1 cardiac progenitors among differentiating cardiac cells from trisomy 21 cells. They uncovered several gene candi-
dates with dysregulated expression including Erg and Ets2. The correlation between chromatin modifications and CCD in trisomy 21 is still to be established.

B. CHARGE Syndrome

CHARGE (coloboma of the eye, heart defects, atresia of the choanae, severe retardation of growth/development, genital abnormalities, and ear abnormalities) syndrome is a complex genetic and epigenetic disease. With an incidence of 1/10,000 live births, cardiac malformations are present in 75% of patients. CHD7, a chromatin remodeling factor (CRF) and a member of the chromodomain helicase DNA-binding family of ATP-dependent chromatin remodeling enzymes, is mutated in CHARGE syndrome patients, which indicates an epigenetic etiology of the disease. Nonsense and frameshift indels are the main mutations that sporadically occur de novo, leading to a loss of function of the protein (17). CRFs including CHD5, CHD6, CDH7, CHD8, and CHD9 participate in the clearance of nucleosomes from regulatory regions of genes to allow for binding of TFs required to turn on transcription. CRFs also play a role in maintenance of heterochromatin. The activation or repressive function of the protein depends on the genomic environment (109, 200). CHD7 is mainly recruited to distal enhancers (197, 264) and associated with H3K4me epigenetic marks (196) pointing to a role of CRF in gene activation.

Several mechanisms may account for cardiac defects in CHD7 mutated patients. CHD7 is required early during cardiac development. Deletion of CHD7 in the Mesp1 mesodermal lineage leads to severe cardiac defects. Semaphorin3c and slit robo pathways are downregulated in mutants, CHD7 also regulates expression of Ca^2+ handling genes, and loss of function affects excitation-contraction coupling of the cardiomyocyte (173).

Furthermore, Nkx2.5 requires CHD7 for its transcription. The CRF interacts with Smad1 and the BMP2 cardiogenic pathway upstream of Nkx2.5 (132). Tbx1, a gene involved in patterning the SHF and found mutated in 22q11 Di-George syndrome, binds ASHL2 (the homolog of the Drosohila homeotic gene absent small homeotic 2). The complex may target CHD7 to Tbx1 binding gene enhancers (222). A loss of function of CHD7 may thus impair the cardiogenic Tbx1 pathway and patterning of the SHF. Animal models may further help understand the molecular mechanisms of the disease. Patten et al. (171) revealed that a knockdown of Chd7 using a morpholino in zebrafish recapitulated part of the CHARGE syndrome phenotype. The zebrafish had severe retina defects and cranial motoneurons. A severe cardiac phenotype was also observed. The heart was dysmorphic in the mutant and featured pericardial effusion (171). The zebrafish might be a good model to decipher the epigenetic basis of this disease as well as to screen drugs such as chromatin modifiers as tentative therapeutic strategies. CHARGE syndrome features were also recapitulated in a mouse from the mouse ENU program that carries CHD7 mutations (26).

C. Cohesinopathies and Mediatoropathies

In multicellular organisms including humans, cell lineage specific enhancers are located at genetic distance from promoters. Furthermore, transcription of major genes governing cell lineage decisions are regulated by super-enhancers located in large open chromatin domains far from promoters (181) and for some of them by pioneer factors that are capable to unlock gene transcription in condensed chromatin. Oct4 and Gatas, both key factors at the crossroad of cardiac cell fate decisions, behave as pioneer factors (1, 219, 263). This has added a combinatorial complexity and specificity to transcriptional regulation. The interaction between distant enhancers and promoters is formed by DNA loops secured by the cohesin and mediator complexes (19).

These DNA loops form three-dimensional structures or sub-TAD (100 kb scale) and TADs (45, 55, 77) (1 Mb scale). TADs have been characterized as replication units (180) and are defined as more conserved structures among cell types, while sub-TADs of smaller sizes are more specific to a cell type (176, 186). Borders of TADs marked by CTCF binding sites are defined computationally from 4C (chromosome conformation capture-on-chip), 5C (chromosome conformation capture carbon copy), or Hi-C, genome-wide 3C (127) data using algorithms. However, these algorithms use thresholding for the relative frequency of interactions within and between loci flanking the TAD borders, which makes the interpretation somewhat subjective. Indeed, the concept of relatively static TADs has recently been challenged, and TADs may be subjected to regulation (246).

In the last decades, genetic diseases have been often associated with mutations or deletions in coding regions of specific genes. However, such an approach has left many genetic diseases without any identified gene (54). This has led to the hypothesis that mutation/deletion in noncoding regulatory regions might also be the origin of diseases by affecting formations of TADs, but this is still minimally investigated. Most importantly, mutations impairing the binding of an enhancer to its promoter or disrupting the borders of TADs, and/or cohesin or mediator loading to DNA within a three-dimensional chromatin structure, will lead to developmental defects as severe as the ones observed in patients affected by a cohesinopathy. This could further explain the variability of phenotypes among patients including trios with an apparent similar genotype. Defective enhancer-promoter interactions could thus be heritable.

Among several groups of cohesinopathies, the Cornelia de Lange syndrome (CdLS) (MIM nos. 122470, 300590,
Cornelia de Lange syndrome phenotypes have been at least in part recapitulated in several mouse models. Heterozygous nipbl−/− mice feature several developmental defects including heart defects such as atrial and ventricular septal defects (194, 265).

Induced pluripotent stem cells derived from CdL patients and differentiated into cardiomyocytes featured NIPBL haploinsufficiency (60–75% of control mRNA level). No significant difference was observed in either the extent of cardiac differentiation or the morphology between wild-type and NIPBL mutated cell lines. However, transcriptomic data revealed a few cardiac genes (GATA4/6, MYH6/7, MYH7, ACTN2, HAND2, TBX1/5, TDGF1) dysregulated in mutated cardiomyocytes. Furthermore, many chromatin modifiers were also found dysregulated. They specifically showed that a change in expression in chromatin-associated complexes (nucleosome and DNA bending), as well as histone modifiers, pointing to an epigenetic impact of NIPBL haploinsufficiency (149).

A novel cohesinopathy was more recently discovered and described as a chronic atrial and intestinal dysrhythmia, termed CAID syndrome. The causative gene is SG01 (old nomenclature: SGOL1), a component of the cohesin complex. The patients suffer from a severe dysregulation of both intestinal and cardiac pacemakers. SGOL1-mutated fibroblasts have a dysregulation of the transforming growth factor-β pathway and display signs of senescence (42). The epigenetic and molecular mechanisms underlying the pathology are under investigation in our laboratories.

Mutations of the components of the mediator complex (MED1 MIM *604311, MED12 MIM *30018) are also at the origin of developmental diseases, called the mediatorpathies including Opitz-Kaveggia syndrome, Lujan-Fryns syndrome, Ohdo syndrome, or MED13L haploinsufficiency syndrome (MIM 309520) (201, 223). Severe cardiac defects are prominent among the developmental disorders affecting Lujan-Fryns syndrome patients. These include TOF (223) (FIGURE 5) and aortic root malformations (252). Other mediatorpathies do not feature any cardiac phenotype. This calls for caution on speculating whether cohesinopathies and mediatorpathies are linked to TAD formation defects.

D. DiGeorge Syndrome

DiGeorge syndrome or 22q11 deletion syndrome is a congenital disease including velocardiofacial and cardiac malformations. The most prominent heart defects are outflow tract malformations and a persistent truncus arteriosus (PTA, due to a lack of septation between the aorta and pulmonary trunk). Candidate genes for the syndrome lie within the q11 region of chromosome 22 that is absent in the genome of patients. Within the locus, Tbx1 has been found as the major gene candidate for the syndrome (14, 15, 129, 172, 187). Haploinsufficiency of Tbx1, a gene predominantly expressed in the SHF, is responsible for outflow tract defects. Specific inactivation of Tbx1 in the anterior heart field (AHF) in the mouse results in a lack of septation between the aorta and pulmonary trunk and expression of pro-differentiation genes (257). This results in PTA and the absence of caudal pharyngeal arches. Besides the genetic role of Tbx1 in the disease, an epigenetic component may play an important role. Early on, the histone chaperone protein HIRA, including WD repeats, was implicated in the 22q11 syndrome (134). HIRA acts on chromatin structure and regulates gene transcription (135). It is a key chaperone for nucleosome assembly (188). Using antisense nucleotide injected into the cardiac neural crest to knock down HIRA in chicken embryos, Farell et al. (65) reported a key role of HIRA in PTA but not in aortic arch defects.

More recently, Tbx1 was found to regulate activity of the histone methyltransferase LSD1 (70) and to exert its action on gene expression through this enzyme. Tbx1 also interacts with the chromatin modifier Barf60a/Smarcd1 and with the Set7 histone H3K4 monomethyltransferase (38). Tbx1 thereby regulates Wnt5 and many other target genes in the SHF through these enzymes.

Several animal models have been used to recapitulate the disease. Piotowski et al. (178) found that the Van Gogh
zebrafish mutant (vgo) featured a DiGeorge syndrome phenotype due to a mutation of tbx1. They showed that vgo/tbx1 acts cell autonomously in the pharyngeal mesoderm and in neural crest-derived cartilages. The fish displayed defects in pharyngeal arches, associated territories, and ear (178). Mouse lines with deletions in a specific region equivalent to human 22q11 were key in determining the role of Tbx1 in the cardiac defects observed in Velo-Cardio-Facial/Di-George syndrome (99, 128, 145). Tbx1 haploinsufficient mice (129) recapitulate most of the phenotypes of DiGeorge syndrome patients including outflow tract defects (13). A recent report from the same laboratory brought further evidence backing the epigenetic hypothesis of DiGeorge syndrome. The authors observed that Tbx1 positively regulates monomethylation of histone 3 lysine 4 (H3K4me1). Tbx1 directly recruits the histone methyltransferase LSD1, an enzyme of the COREST complex. Such recruitment is lacking in Tbx1 haploinsufficient mice. Importantly, the treatment of mice with an LSD1 inhibitor rescued part of the cardiac phenotype (70).

These data reveal that DiGeorge syndrome features many epigenetic components and suggest that the syndrome in a more complex disease than expected.

**FIGURE 5** illustrates the main cardiac malformations in TOF and (epi)genetic diseases.

**VI. CONCLUSIONS**

We have overviewed the basic knowledge of epigenetic regulation of heart development. We further described cardiac congenital diseases associated with epigenetic dysfunction.

Cardiac congenital diseases show that genetics turns out to be more complex than thought. In many cases, a single gene mutation cannot explain a given patient’s phenotype. For example, it is still unclear how a slight decrease in gene expression is responsible for a severe phenotype. This suggests additional mechanisms including dysfunction in transcriptional factory formation, defects in super-enhancer activation, or aberrant three-dimensional chromatin structure.

Building of the transcriptional factors within the cell nucleus is still a poorly understood process. How transcription factors and chromatin remodelers or transcription modulators associate at a specific locus is still the subject of intensive research. Super-enhancers have not been fully mapped within the genome. Why a gene besides constitutive genes needs a super-enhancer is a question that remains to be elucidated. How super-enhancers are activated by multiple transcription factors at once on a timely basis is still questionable.

While we have significantly learned about the formation and maintenance of chromatin architecture, there is still a lot of information missing on the dynamics and plasticity of this scaffold when cells divide, stop dividing, or change fate. Adaptation of chromatin structure to the function of the cell is likely to be a sensitive step prone to dysfunction and a potential origin for diseases.

A currently emerging field of research includes the effect of nutrition of the mother on epigenetic modulation of gene transcription in offspring. A recent paper illustrates this topic by reporting the effect, in mouse, of maternal diabete-induced hyperglycemia of offspring (18). Hyperglycemia leads to an haploinsufficiency of Notch, inhibited by Jarid2, a regulator of histone methyltransferase.

Epigenetics and developmental biology are still fast-moving fields of research. While we know the basis of many mechanisms, a long road ahead will teach us about the fine tuning of the latter. Undoubtedly, this will reveal the origin of many cardiac congenital diseases.

**ACKNOWLEDGMENTS**

T. Moore-Morris and P. P. van Vliet contributed equally to this work.

Address for reprint requests and other correspondence: M. Puceat, Université Aix-Marseille, INSERM UMR-1251, Marseille, France (e-mail: michel.puceat@inserm.fr).

**GRANTS**

We acknowledge the Leducq Foundation for the long-standing support of Puceat’s laboratory.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


Dixon JR, Jung I, Selvaraj S, Zhu J, Roysam B,下一节

EPIGENETICS


121. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene. _Cell_ 59: 8674(92)90611-F.


229. Takeuchi T, Kojima M, Nakajima K, Kondo S. jumonji gene is essential for the neuration of chromatin domains and compartments in single chromosomes.


229. Takeuchi T, Kojima M, Nakajima K, Kondo S. jumonji gene is essential for the neuration of chromatin domains and compartments in single chromosomes.


229. Takeuchi T, Kojima M, Nakajima K, Kondo S. jumonji gene is essential for the neuration of chromatin domains and compartments in single chromosomes.


