The processes that govern the growth and differentiation of a single-cell fertilized zygote to an organism that contains multiple organs and various cell types with a diverse array of functions remain largely enigmatic. A tantalizing aspect of this process is that the genome, with the exception of rare mutations introduced during DNA replication, is identical among the various cell types that form an organ or an organism. There are, however, subtle differences in the DNA sequence among cells because of the rare error rate of DNA polymerases and editing enzymes responsible for the replication of the genome. The error rate, which is estimated to be $\approx 1 \times 10^{-8}$, introduces $\approx 30$ de novo mutations per meiosis. In addition, single-cell whole-transcript sequencing indicates the presence of considerable cell-to-cell variability in the transcript copy number and splicing among different cells of the same type within an organ. Thus, no 2 cells in an organism are totally identical. Yet, cells seem to benefit from a higher order of regulation that affords them lineage specification and differentiation into groups of cells with similar structural and functional characteristics.

The discovery of the transcription factors (TFs) provided a major clue to the understanding of lineage specification during development. Approximately 10% of the genes in the human genome encode TFs, which reflects the biological significance of the TFs. In the muscle, myogenic differentiation was the first TF that was identified and was shown to have the potential to induce myogenesis on ectopic expression in the fibroblasts. Subsequently, several other muscle TFs, such as myogenin and myogenic factor 5, were cloned and characterized. In the heart, NK2 homeobox 5 (cardiac specific homeobox 1 or tinman) and islet 1 were among the first set of TFs that were identified and characterized. Thus far, $>2$ dozen TFs that regulate the differentiation, proliferation, and lineage specification of cardiac progenitor cells have been identified and characterized. Notable features of the TFs are cardiac regional expression and functionality, which enable migration and differentiation of the embryonic cells in a manner that commits them to different cardiac regions and cell types. Furthermore, TFs function cooperatively to modify the chromatin, assemble to the promoter regions, and regulate the expression of their target genes. In line with the regional expression, TFs that regulate the first or anterior heart field, which gives rise to the left ventricle, differ from those that regulate the second heart field, the source of the right ventricle and the outflow tract. Temporal and spatial expression of the TFs is a major determinant of cell lineage specification and patterning of the heart. Consequently, it is not surprising that mutations in the TFs or their coregulator proteins impair cardiac development and lead to congenital heart defects. Among the best-known TFs implicated in the pathogenesis of congenital heart defects is NKX2.5 or CSX1, because mutations in NKX2.5 are associated with atrial and ventricular septal defects and tetralogy of Fallot. Similarly, mutations in T box 5 and GATA binding protein 4 are known to cause the Holt–Oram syndrome and tetralogy of Fallot, respectively.

Genes occupy only $\approx 1.5\%$ of the genome. However, $\leq 15\%$ of the genome is under evolutionary constraint, indicating the presence of DNA elements with biological functions. The findings of the Encyclopedia of DNA Elements (ENCODE) project provide a blueprint of the regulatory elements in the genome, which also entail the enhancers. In contrast to TFs, which regulate the gene expression by directly binding to the specific DNA sequences in close proximity to the coding regions (promoter regions), enhancers are cis-regulatory elements that are often not located in close proximity to the coding sequences but regulate gene expression in a cell type–specific manner during organogenesis. Enhancers are much more abundant in the genome than the TF-encoding genes. Recent genome-wide mapping of the enhancer in the human fetal and adult hearts identified $\approx 6200$ candidate enhancer sequences in the genome. In addition to being abundant,
enhancers also exhibit dynamic regulation, typically through epigenetic modifications of histones. Specific methylation of histones demarcates the silent and active enhancers.\textsuperscript{45–47} The significance of the enhancers in regulating cardiac development is reflected by the recent identification of the de novo mutations in the genes that code for the histone-modifying enzymes in patients with sporadic forms of congenital heart defects.\textsuperscript{48}

Beyond regulating the silenced, chromatin remodeling, either through DNA methylation or through post translational modifications of histones or the ATP-dependent chromatin changes, plays a major role in regulating gene expression during cardiac development as well as in the pathological states.\textsuperscript{25,26,49,50} Epigenetic mechanisms regulate the development of all cardiac lineages, including myogenesis and angiogenesis.\textsuperscript{45,51–53} Epigenetic marking of the chromatin through trimethylation of histone H3 on lysine 27 represses gene expression, whereas histone 3, lysine 4 trimethylation activates gene expression. Among the notable characterized epigenetic complexes is the polycymb repressive complex 2 that silences inappropriate gene expression during cardiac development on histone H3 on lysine 27 marking of the chromatin.\textsuperscript{51} Similarly, histone deacetylation regulates various developmental events in the cardiovascular system, such as smooth muscle cell differentiation.\textsuperscript{51} The epigenetic mechanisms not only tightly regulate lineage commitment of the progenitor cells but also regulate the stemness itself.\textsuperscript{52}

Cardiac development is further fine-tuned by noncoding RNAs. Prominent members of this class are the long noncoding RNAs and microRNAs (miRs).\textsuperscript{54–59} Despite the nascent field, the long noncoding RNAs Braveheart and FOXF adjacent non-coding developmental regulatory RNA have been shown to regulate cardiogenesis.\textsuperscript{60,61} The Braveheart seems to function upstream of the mesoderm posterior 1 TF to regulate the activation of cardiovascular genes.\textsuperscript{60,62} The long noncoding RNA Braveheart also interacts with the polycymb repressive complex 2 complex and links the non-coding RNAs to epigenetic regulation of cardiac development.\textsuperscript{60} Similarly, the long noncoding RNA forkhead box F1 adjacent to noncoding developmental regulatory RNA (FENDRR) regulates several TFs involved in cardiac mesoderm differentiation.\textsuperscript{63} FENDRR seems to exert its effects on cardiogenesis also through binding to the polycymb repressive complex 2 and the trithorax group/mixed lineage leukemia complex and, hence, modifying the chromatin signatures and influencing gene expression.\textsuperscript{64} In addition, several miRs have been implicated in cardiovascular development and differentiation of cardiac progenitor cells.\textsuperscript{7,63–72} The miR-15 family has been implicated in postmitotic arrest of cardiac myocytes.\textsuperscript{73} Similarly, miR-23 is involved in the regulation of endocardial cushion formation during embryonic heart development.\textsuperscript{70} Furthermore, a combination of miRNAs 1, 133, 208, and 499 has been shown to induce direct reprogramming of fibroblasts to cardiac myocyte–like cells in vitro.\textsuperscript{65,74–76}

Complex regulatory networks coordinate interactions among the TFs, enhancers, epigenetic chromatin remodeling factors, and noncoding RNAs, as well as other constituents that regulate cardiogenesis. The regulatory complex also uses various signaling pathways, notable among them are the canonical Wnt signaling pathway,\textsuperscript{51,77–82} Notch signaling pathway,\textsuperscript{83–85} and others,\textsuperscript{51,86,87} to regulate cell proliferation, migration, and differentiation during cardiac development. In summary, cardiogenesis involves proliferation, migration, and differentiation of the progenitor cells in response to a series of complex, coordinated, dynamic, and stochastic biological regulators that guide the development of 3-dimensional heart structures.

Disclosures

None.

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