The adult mammalian heart has a limited capacity for replacing lost tissue following injury. Mammalian cardiomyocytes exit the cell cycle shortly after birth, where they undergo a final round of DNA synthesis, binucleate, and terminally differentiate. In addition, low levels of cardiomyocyte turnover in the aging heart or following injury seem to be mediated mostly by cardiomyocyte division. Although the neonatal mammalian heart can regenerate following injury, this regenerative capacity is lost by the end of the first week of life, with cessation of cardiomyocyte proliferation. Thus, a deeper understanding of mechanisms of cardiomyocyte cell cycle regulation is a critical step toward harnessing the endogenous regenerative capacity of the adult heart.

One of the lasting enigmas in the cardiovascular field has been the mechanism, and perhaps more importantly the reason, for the permanent cell cycle arrest of the majority of cardiomyocytes in the postnatal heart. These questions only become more pressing when comparing the injury response of the neonatal and adult hearts.

We recently identified a member of the TALE homeodomain transcription factors, Meis1, as an important regulator of postnatal cardiomyocyte cell cycle arrest. Although Meis1 mRNA was only modestly dysregulated during the narrow postnatal regeneration window, we found that Meis1 nuclear translocation corresponded to cardiomyocyte cell cycle exit. Myocyte-specific Meis1 deletion resulted in extension of the postnatal proliferative window in the neonatal heart and, to a lesser extent, reactivation of myocyte proliferation in the adult heart. In contrast, Meis1 overexpression was sufficient to reduce cardiomyocyte proliferation and inhibition of neonatal heart regeneration.

Progression of eukaryotic cells through the cell cycle is controlled by the specific activation of a series of CDKs and cyclins. Cyclin/Cdk complexes are regulated by 2 families of CDK inhibitors, the INK4 family (including p15, p16, p18, and p19) and the CIP/KIP family (including p21, p27, and p57). p16 and p21 are CDK inhibitors that play key roles in differentiation and in cell cycle arrest. Simultaneous induction of p21 and p16 expression cooperatively blocks the activation of CDK4/6 and CDK2, the key regulators of G1/S and G2/M checkpoints. p16 also represses the transcription of Cdc20, a key regulator of spindle assembly checkpoint. Therefore, simultaneous regulation of both p16 and p21 will affect cardiomyocyte cell cycle at all 3 checkpoints. Examination of Meis1-null cardiomyocytes showed significant downregulation of CDKIs from both the Ink (Ink4b/Arf/Ink4a) and the CIP/KIP families. We further showed that Meis1 is an important transcriptional activator of p16 and p21 expression and that p21 inhibition will affect cardiomyocyte cell cycle at all 3 checkpoints.

Despite this exciting new perspective on transcriptional regulation of cardiomyocyte cell cycle, there are several questions that remain unanswered. First, the diverse and complex role that Meis1 plays in different cell types is likely closely related to the local environment as well as the expression pattern of Meis1 cofactors. We have previously shown that loss of Meis1 in hematopoietic stem cells (HSCs) results in loss of quiescence and increased apoptosis secondary to downregulation of the hypoxia master regulators Hif-1α and Hif-2α. While the upregulation of Hifs may be critical for the survival of HSCs in their hypoxic environment, it may have less of an effect in highly oxidative cardiomyocytes under normal conditions, which precludes stabilization of Hif transcription factors. In support of this notion, we observed no change in apoptosis following conditional deletion of Meis1 in cardiomyocytes. It is important to note here that while Hifs are generally degraded in cardiomyocytes under normoxic conditions, myocardial ischemia stabilizes Hifs, which, in turn, play a critical role in stress response of cardiomyocytes.

While the transcriptional activity of Meis1 in HSCs is closely associated with Hoxa9 and Pbx1, the expression pattern of Meis1 cofactors in cardiomyocytes appears to be quite different. We did not detect significant Hoxa9 or Pbx1 expression in postnatal cardiomyocytes, while the only Meis1 cofactors upregulated > 2-fold between p1 and p7 (out of 37 Hox factors tested) were Hoxa10, Hoxa11, and Hoxd12. Intriguingly, these 3 factors, along with Hoxa9 and Hoxb13 (which was only modestly upregulated), are the only confirmed Hox factors that interact with Meis1. Therefore, it appears that the distinct pattern of expression of Meis1 cofactors is a critical determinant of its activity as redox and cell cycle regulator. It will be imperative for future studies to understand the role of Meis1 in the local environment and how this affects its transcriptional activity in cardiomyocytes.
outline the specific role of Meis1 cofactors in regulating its diverse expression pattern and function and, more importantly, to outline the upstream events that regulate the specific expression pattern of Meis1 and its cofactors (Fig. 1).

Other considerations of cardiomyocyte cell cycle regulation may relate to specific contractile properties of cardiomyocytes. A hallmark feature of proliferative neonatal cardiomyocytes is sarcomere disassembly, where sarcomeric structures undergo degradation and localization to the periphery of the myocytes. It is unclear how this affects the contractility, or whether the isoform of sarcomeric proteins (neonatal vs. adult) plays a role in their susceptibility to degradation during mitosis.

There is no doubt that the cardiac regeneration field has advanced rapidly over the past few years. There is now a clear view that targeting cardiomyocyte cell cycle is a viable therapeutic target, and Meis1 may prove to be an important piece of that puzzle. Regardless of the specific molecular mechanism that mediates postnatal cell cycle arrest of cardiomyocytes, one has to wonder: Why do the majority of cardiomyocytes exit cell cycle permanently after birth? Is it a protective mechanism against malignant transformation? Is it an inherent property of an incessantly contractile organ? The key to unlocking the regenerative ability of the adult heart may lie in integrating these conceptual and mechanistic questions.

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Figure 1. The diverse roles of Meis1. In the bone marrow, Meis1 and its cofactors transcriptionally activate Hif-1α and Hif-2α, leading to a reduction in the level of reactive oxygen species and maintenance of hematopoietic stem cells (HSC) quiescence (left panel). In the postnatal heart, Meis1 (and perhaps its cofactors Hoxa10, Hoxa11 and Hoxd12) transcriptionally activates CDKIs, thus contributing to the postnatal cell cycle arrest of cardiomyocytes (right panel).