Iscemic diseases and heart failure remain the major causes of morbidity and mortality in the industrialized world. Several new therapeutic modalities have been developed to interfere with the response to injury and to improve cardiac function after ischemia or cardiomyopathy. Experimental and clinical studies demonstrated that the transplantation of bone marrow–derived or tissue-resident stem/progenitor cells in diseased hearts improves neovascularization and functional recovery.1 However, the recent discovery of cardiac stem cells (CSCs) in the adult heart poses the question whether activation of the endogenous pool of these resident stem cells may compensate for the loss of cardiac tissue after injury and improve functional recovery. Indeed, a genetic fate-mapping study elegantly demonstrated that stem cells or precursor cells significantly contributed to the replacement of adult mammalian cardiomyocytes after injury.2 Although it remains unclear to what extent cardiac regeneration in this genetic model was mediated by CSCs or circulating progenitor cell populations being attracted to the heart after injury, mounting evidence suggests that injury-associated signals activate CSCs in situ, which subsequently contribute to the refreshment of the injured heart.3 Obviously, to play a significant role in endogenous cardiac regeneration, the relatively small number of endogenous CSCs needs to be expanded after injury before differentiating into cardiac myocytes. However, the molecular mechanisms underlying the activation, expansion, and recruitment of resident CSCs after injury remain unclear.

In the current issue of Circulation Research, Siddiqi et al provide an intriguing concept, which might help to mechanistically explain how proliferation of CSCs is regulated in response to injury.4 The authors investigated the cardiac expression of stem cell specific genes, involved in self-renewal and proliferation, during cardiomyopathic injuries and found increased expression of the nucleolar protein nucleostemin—recently discovered in embryonic and adult stem cells as well as in cancer cell lines.5 Detailed expression analysis in the heart revealed that nucleostemin protein was expressed in the heart at birth but rapidly declined in the first weeks of postnatal life.4 However, nucleostemin was transiently reexpressed in the adult heart after acute myocardial infarction and other pathological challenges, such as pressure overload (Figure 1A). Interestingly, the transient reexpression of nucleostemin in c-kit+ CSCs may have contributed to their expansion after injury. Indeed, forced overexpression of nucleostemin in cultured CSCs significantly increased that proliferation, whereas cultured CSCs downregulated the expression of nucleostemin during cardiac differentiation (analogous to previous results in other adult stem cells5). Consistent with the concept that only a transient signal is required for expanding the stem cell pool, nucleostemin was expressed in vivo at maximum levels around 72 hours after injury and returned to baseline levels within days.4 Altogether, these data support the concept that a transient upregulation of nucleostemin promotes the proliferation of CSCs, whereas subsequent downregulation of its expression occurs before terminal differentiation. Obviously, these data are based on associations, and the causal role of nucleostemin for endogenous cardiac regeneration needs to be further tested in genetic animal models.

Surprisingly, the expression of nucleostemin was not only increased in the c-kit+ CSCs, but also in selective cardiomyocytes after myocardial infarction or pressure-overload hypertrophy.4 The reexpression of nucleostemin in cardiomyocytes reminds to the well-established reactivation of the embryonic gene program during pathological hypertrophy; however, the functional relevance of this reexpression remains unknown. Indeed, in contrast to CSCs, forced overexpression of nucleostemin in cardiomyocytes failed to induce proliferation, and alternative functions of nucleostemin in cardiomyocytes were not studied. Generally, the molecular mechanisms of nucleostemin-mediated functions are not entirely clear. The protein consists of an N-terminal basic domain, which specifies nucleolar localization, and 2 GTP-binding motifs, which are involved in its interaction with p53.5 Removal of the GTP-binding motifs was shown to induce p53-dependent apoptosis indicating that nucleostemin might inhibit p53 activity.3 However, forced overexpression of nucleostemin in cardiomyocytes was insufficient to antagonize p53.4 Nucleostemin was also reported to delay cellular senescence in mouse embryonic fibroblasts.6 Consistent herewith, in CSCs, forced overexpression of nucleostemin resulted in the absence of telomere shortening despite increased prolifera-
mediated increase in nucleostemin is consistent with previous studies in bone marrow stromal cells; one wonders whether other angiogenic factors such as VEGF, PDGF, and PDGFs might also exert effects on adult heart regeneration through modulation of nucleostemin expression. Interestingly, upregulation of nucleostemin was detected in Pim-1 transgenic mice. The kinase Pim-1 is activated via Akt and protects against cardiomyocyte apoptosis. Although the causal sequence of events has not been experimentally proven, one may speculate that growth factor-mediated activation of Akt and subsequent stimulation of Pim-1 may have resulted in augmented nucleostemin expression (Figure 1B). The molecular link between Pim-1 and nucleostemin deserves further investigation, yet a direct interaction between the 2 proteins seems unlikely as no colocalization of Pim-1 and nucleostemin was detected on histological sections of the Pim-1 transgenic hearts. In addition, an outstanding issue remains the identification of the injury-associated molecular signals stimulating the expression of nucleostemin in CSCs.

The authors’ findings on nucleostemin expression and function in CSCs might have implications for our understanding of the regulation of cardiac stem cell fate upon injury. Indeed, the pivotal role of nucleostemin expression in proliferation versus differentiation of CSCs resembles that of c-myc expression in the early fate decisions of hematopoietic stem cells. Thus, while lowly expressed in quiescent cells residing in the putative cardiac stem cell niches, (so far unidentified) injury-associated signals activate the cardiac stem cell pool and induce the expression of nucleostemin, thereby promoting cardiac stem cell expansion at the expense of differentiation (Figure 2). Intriguingly, however, and likely in contrast to the c-myc report, nucleostemin-expressing CSCs in injured hearts were located outside of their normal quiescent microdomains. Indeed, after myocardial infarction, the authors identified nucleostemin-expressing cKit+ CSCs in the ischemic border zone and not in the atria or apex, which were previously proposed to be the main locations of cardiac stem cell niches. Hence, one might speculate that, in response to ischemic injury, activated CSCs first leave their normal quiescent niches and translocate to other niches, more permissive for increased expression of nucleostemin and cardiac stem cell expansion (putatively termed “cardiogenic niches”; Figure 2). If true, then the identification of the molecular signature of these cardiogenic niches might provide further insights how to increase the contribution of CSCs during regeneration.

**Figure 1.** Regulation and proposed function of nucleostemin in the heart. A, Induction of nucleostemin in different models. B, Potential mechanism of growth factor-mediated upregulation of nucleostemin. C, Effect of nucleostemin on the CSCs.

**Figure 2.** Putative model for the role of nucleostemin in the fate determination of adult cardiac stem cells (CSCs) in response to injury-associated molecular signals. Unidentified activation signals might translocate quiescent C-kit+ CSCs to a “cardiogenic niche” and induce the expression of nucleostemin (NS) in CSCs, resulting in CSC proliferation without the risk of telomere shortening (through increased expression of TERT, TRF1, and TRF2). In a second phase, unidentified differentiation signals might halt CSC proliferation by switching off the expression of nucleostemin while promoting the differentiation to C-kit+ cardiomyocytes.
adult heart regeneration. It will also be interesting to find out whether established stimulators of cardiac differentiation (eg, TGF/BMP, Wnt, Notch and Sox pathways) are capable of switching off the expression of nucleostemin in the expanded cardiac stem cell pool, thereby completing the cardiac regeneration program.

In summary, the study by Siddiqi et al provides novel insights into the regulation and function of nucleostemin in the heart. Although the in vivo function of nucleostemin needs to be confirmed, the results tempt to speculate that nucleostemin might be crucial for the proliferative response of CSCs and cardiomyocytes after injury. However, it remains unclear why the induction of nucleostemin expression after injury and by pathological challenges is not activating endogenous cardiac regeneration to an extent sufficient to prevent the further development of cardiac dysfunction and heart failure. One may speculate that activation by an additional pathway would further enhance the repair after injury. In this respect, the identification of pathways to upregulate nucleostemin expression such as the FGF2/Akt/Pim-1 axis offers novel therapeutic options to promote adult heart regeneration. An additional level of complexity, however, has to be taken into account, as nucleostemin localization and GTP-driven shuttling between the nucleolus and nucleoplasm is tightly regulated and its functional activity critically depends on the correct subnuclear localization. The elucidation of the complex regulation of mobilization, proliferation, and differentiation of resident CSCs and cardiomyocytes under physiological and pathophysiological conditions thus warrants further investigations with the option to develop new therapeutic strategies to augment endogenous repair for preventing or treating heart failure.

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