Caught Red-Handed
Cycling Cardiomyocytes
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During the past couple of years, overwhelming evidence has accumulated that the myocardium in adult mammals including humans undergoes significant renewal.\(^1,2\) The underlying homeostatic renewal process enables the heart to partially replace essential building blocks during a lifetime but is not sufficient to cope with the increased loss of cardiomyocytes under acute or chronic pathological conditions. Although good reasons might exist why nature has restricted replacement of cardiomyocytes in adult mammals, enhancement of the physiological renewal rate by therapeutic interventions might provide means to unearth hidden regenerative capacities and restore lost cardiac functions.

At present, no consensus exists about the source of cells enabling homeostatic renewal of the myocardium in adult mammals. New cardiomyocytes might originate from rare divisions of existing cardiomyocytes, probably after moderate or extensive cardiomyocyte dedifferentiation, from putative cardiac stem cells, or from a combination of both. Several attempts have been made in the past to monitor cardiomyocyte division in the developing and adult myocardium, clearly defining the major phases of cardiomyocytes proliferation.\(^3\) However, reliable detection of rare cell cycling events in adult cardiomyocytes proved to be difficult, mostly because of the tightly packed arrangement of multiple cell types in the myocardium and the abundance of noncardiomyocytes, which proves to be difficult, mostly because of the increased loss of cardiomyocytes under acute or chronic pathological conditions.

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antibodies. Therefore, other researchers have integrated different read-outs (ie, identification of a specific cell type and monitoring of cell cycle activity) into a single system by using cardiomyocyte-specific promoters driving the expression of cell cycle markers, which provided a detailed map of cell cycle dynamics during development in living cardiomyocytes.\(^5,7\)

Hirai at al\(^8\) have now come up with another simple and robust system to monitor S- to M-phase cell cycle activity in murine cells. The approach relies on the conditional activation of a cyclinA2–enhanced green fluorescent protein (eGFP) fusion gene by expression of Cre-recombinase. In their study, Hirai at al\(^8\) have used a Protamine-Cre line to activate the cyclinA2–eGFP fusion gene in the germ line, which allows assessment of cell cycle activity in all cells. In addition, the authors have taken advantage of a cardiac myocyte–specific troponin T-Cre recombinase (TnT-Cre) transgene to specifically activate the reporter gene in cardiomyocytes. The new tool was validated by comparing cell cycle–specific expression of eGFP to incorporation of the thymidine analogue 5-ethyl-2’-deoxyuridine (EdU) and appearance of the M-phase marker phosphohistone H3, which mark S and M phase, respectively (Figure [A]). Importantly, cyclinA2–eGFP expression was completely lost within 6 to 12 hours after synchronized embryonic fibroblasts were forced into cell cycle arrest (G0) by serum starvation. This experiment convincingly demonstrated that the cyclinA2–eGFP fusion protein is rapidly degraded during M phase and therefore not carried over from one cell cycle to the next. In the TnT-Cre×cyclinA2–eGFP transgenic mouse ≈80% of all cyclinA2–eGFP positive cardiomyocytes incorporated EdU when a single EdU injection was given to the animals 2 or 4 hours before the analysis. This finding was expected because EdU is only incorporated during S phase, whereas cyclinA2–eGFP is already expressed at mid-G1. Analysis of the mitotic activity of cardiomyocytes at different developmental stages with the new technique confirmed previously published results reporting a gradual decrease of cardiomyocyte proliferation with progressive development including a major decline of mitotic activity in the early postnatal period. Furthermore, the authors made several new observations. First (and probably most important), Hirai at al\(^8\) did not find evidence for a proliferative burst of ventricular cardiomyocytes at postnatal day 15, which was claimed recently in a hotly debated publication in Cell by Naqvi et al\(^9\) to establish the final cardiomyocyte number in mouse hearts. Instead, Hirai at al\(^8\) detected a further gradual and steady decline of mitotic activity from postnatal day 14 to 20, which eventually resulted in a nearly complete cessation of cell cycle activity in the first 3 weeks after birth. Recently, Alkass et al\(^10\) and Soonpaa et al\(^10\) published similar results using the above-mentioned pericentriolar material 1/5-bromo-2’-deoxyuridine.
might provide a more reliable approach to detect dividing cardiomyocytes although Hesse et al. used an ubiquitous protein anillin as recently described by Hesse et al. Anillin is located in the nucleus during G1, S, and G2 phase, in the cytoplasm in early M phase, and in the contractile ring in cytokinesis. Anillin is activated by troponin T-Cre recombinase (TnT-Cre). Combination of CAG–Anillin–eGFP (green) and β-myosin heavy chain promoter-dependent hH2B-mCherry expression (red) allows identification of cardiomyocytes in G0, G1 to G2, karyokinesis, and cytokinesis. Anillin leaves room for further improvement. cyclinA2 expression marks the S to M phases of cell cycle but does not necessarily indicate cytokinesis. In fact, Hirai at al disclosed a previously unrecognized lower rate of cardiomyocyte cell cycle activity after birth at P0 and P1 in the right when compared with the left ventricle. The early cell cycle exit in the right ventricle might, at least in part, contribute to differences in ventricular wall thickness that emerge during postnatal development. However, additional studies are clearly required to validate this finding and explore its significance.

The new tool developed by Hirai et al provides many advantages when compared with traditional approaches such as ease of use, dependence on a single fluorescence marker, high reliability, and superior sensitivity when compared with EdU incorporation. In addition, potential adverse effects of BrdU or EdU incorporation can be ruled out and noise generated by incorporation of labeled nucleosides during DNA repair. On the contrary, a couple of limitations is evident, which leaves room for further improvement. cyclinA2 expression marks the S to M phases of cell cycle but does not necessarily indicate cytokinesis. In fact, Hirai at al observed cyclinA2–eGFP activity during cell cycle events in the early postnatal period, which preceded binucleation. In other words, the cyclinA2–eGFP reporter will label cardiomyocytes that undergo binucleation and most likely also endoreduplication but not necessarily commit to cytokinesis. Visualization of midbody formation in cardiomyocytes using eGFP fused to the scaffold protein anillin as recently described by Hesse et al. might provide a more reliable approach to detect dividing cardiomyocytes although Hesse et al. used an ubiquitous promoter to drive expression of the EGFP–anillin fusion and did not attempt to restrict the expression to the cardiomyogenic lineage. However, combination of the CAG–anillin–eGFP line with mice that express a fusion protein of human histone 2B and the red fluorescence protein mCherry under the control of the β-myosin heavy chain promoter allows trustworthy detection of dividing cardiomyocytes (Figure [B]).

Activation of the cyclinA2–eGFP reporter relies on the cell type–specific expression of Cre-recombinase, which makes it difficult to combine the technique with other Cre-recombinase–based labeling approaches. For example, Kimura et al have recently devised a clever system for fate mapping of hypoxic cardiomyocytes, which are present at low numbers in the adult heart but contribute widely to formation of new cardiomyocytes. Obviously, it would be highly useful to label hypoxic cardiomyocytes and simultaneously monitor cell cycle activity in real time. The use of different recombinases like Flp and Cre that do not interfere with each other might overcome such compatibility issues and combine the benefits of both approaches. Finally, the cyclinA2–eGFP reporter described by Hirai et al does not allow live cell tracing and visualization of different phases of the cardiomyocyte cell cycle, which was possible with a recently described multicolor fluorescent ubiquitination-based cell cycle indicator reporter. Fluorescent ubiquitination-based cell cycle indicator was first described in 2008 by Sakaue-Sawano et al and exploits the regulation of cell cycle–dependent ubiquitination to effectively label individual cell nuclei in G1 phase in red, and those in S/G2/M phases in green (Figure [C]). On the contrary, the current fluorescent ubiquitination-based cell cycle indicator system is also not without disadvantages, namely, the low florescence intensities of green/red reporters in different cell types, which makes live cell imaging of rarely dividing cardiomyocytes a challenge. A careful selection of the reporter or assay systems will always be necessary to fit the specific experimental needs and the underlying scientific question. Time will tell whether future cell cycle reporters can combine all features of the above-described systems and qualify as a Jack-of-all-trades.

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**Disclosures**

None.

**References**


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