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 myocardium4 and the abundance of noncardiomyocytes, which constitute 60% of all cell nuclei in the neonatal mouse heart and ≈70% in young adult mice.5 Pioneering research from Loren Field’s group has used genetic labeling of cardiomyocytes in mice using an myosin heavy chain-nuclear localized protocadherin1aperted Cre reporter transgene in combination with tritiated thymidine incorporation to overcome this problem,6 whereby others have used antibodies against pericentriolar material 1 as an accurate marker to label human and murine cardiomyocyte nuclei.6 However, such approaches are either cumbersome and time-consuming or rely on the use of multiple 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 al8 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 al8 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-ethynyl-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 al8 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 al9 to establish the final cardiomyocyte number in mouse hearts. Instead, Hirai at al8 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 al10 and Soonpaa et al10 published similar results using the above-mentioned pericentriolar material 1/5-bromo-2’-deoxyuridine.

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might provide a more reliable approach to detect dividing cardiomyocytes although Hesse et al.11 used an ubiquitous folding protein anillin as recently described by Hesse et al.11 formation in cardiomyocytes using eGFP fused to the scaffold protein anillin. 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 located in the nucleus during G1, S, and G2 phase, in the cytoplasm in early M phase, and in the contractile ring in cytokinesis. In fact, Hirai et al.8 observed cyclinA2–eGFP activity during cell cycle events in the early postnatal period, which preceded binucleation. In other words, the cycle–specific degradation of cyclinA2–eGFP (green) and α-myosin heavy chain promoter–dependent H2B-mCherry expression (red) allows identification of cardiomyocytes in G0, G1 to G2, karyokinesis, and cytokinesis. 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. Cdt1-Kusabira Orange levels are highest in G1, whereas Geminin-Azami Green levels are highest during the S/G2/M (green) phases. FUCCI exploits the cell cycle–dependent ubiquitination and degradation of Cdt1-Kusabira Orange and Geminin-Azami Green. Cdt1-Kusabira Orange levels are highest in G1, whereas Geminin-Azami Green levels are highest during the S/G2/M phases. Cre-dependent activation of FUCCI in the Rosa26 locus generates cell type specificity.

Figure. Schematic comparison of different cardiomyocyte cell cycle reporter systems in mice. A, Cell cycle–specific degradation of the cyclinA2–eGFP fusion protein (eGFP) fusion protein (green) allows labeling of cardiomyocyte nuclei from S to M phase. Expression of the cyclinA2–eGFP fusion protein starts in mid-G1 phase, peaks in S phase, and fades out in mitosis. Expression in cardiomyocytes is activated by troponin T-Cre recombinase (TnT-Cre). B, Combination of CAG–Anillin–eGFP (green) and α-myosin heavy chain promoter–dependent H2B-mCherry expression (red) allows identification of cardiomyocytes in G0, G1 to G2, karyokinesis, and cytokinesis. 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. C, The multicolored fluorescent ubiquitination-based cell cycle indicator (FUCCI) system allows identification of cells in G1 (red) and in S/G2/M (green) phases. FUCCI exploits the cell cycle–dependent ubiquitination and degradation of Cdt1-Kusabira Orange and Geminin-Azami Green. Cdt1-Kusabira Orange levels are highest in G1, whereas Geminin-Azami Green levels are highest during the S/G2/M phases. Cre-dependent activation of FUCCI in the Rosa26 locus generates cell type specificity.

(A) CyclinA2-TnT-Cre x TnT-Cre

(B) CAG-Anillin-eGFP x α-myosin H2B-mCherry

(C) R26FucciORX Cardiomyocyte-specific Cre

(1) CyclinA2-TnT-Cre x TnT-Cre

(2) CAG-Anillin-eGFP x α-myosin H2B-mCherry

(3) R26FucciORX Cardiomyocyte-specific Cre

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Disclosures

None.

References


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