Survey of Studies Examining Mammalian Cardiomyocyte DNA Synthesis

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Increases in cardiac mass during fetal life arise predominantly as a consequence of cardiomyocyte proliferation. During neonatal life, there is a transition from hyperplastic to hypertrophic growth such that further increases in myocardial mass are typically not accompanied by cardiomyocyte proliferation. In the adult myocardium, it is generally believed that the vast majority of cardiomyocytes do not proliferate. This view is supported in part by clinical observations: functionally significant myocardial regeneration has not been documented in diseases and/or injuries that result in cardiomyocyte loss. Furthermore, primary myocardial tumors are rarely observed in adults.

Although these findings suggest that the proliferative capacity of adult cardiomyocytes is quite low, they do not exclude the existence of a limited degree of hyperplastic growth in either the normal or diseased myocardium. Toward this end, a number of studies examining the proliferative capacity of cardiomyocytes in experimental animals have been reported. Because genome reduplication is a prerequisite for cell proliferation, the majority of these studies have used various methodologies to monitor cardiomyocyte DNA synthesis as a first approximation of cell division. In the present survey, issues that we consider pertinent for accurate assessment of cardiomyocyte DNA synthesis are discussed. The literature examining cardiomyocyte DNA synthesis during normal and pathological myocardial growth is then summarized.

Assessment of Cardiomyocyte DNA Synthesis In Vivo

Accurate assessment of cardiomyocyte DNA synthesis in vivo is dependent on the selection of an appropriate marker for genome reduplication as well as the criteria used for cardiomyocyte identification. These issues are considered separately below.

Markers for DNA Synthesis

Genome reduplication is accompanied by a variety of cytological, biochemical, and molecular events, many of which can be used as either direct or indirect evidence for DNA synthesis and, by inference, proliferation. These include the presence of cytological landmarks indicative of karyokinesis, the presence of active DNA synthesis, and the expression of genes and/or protein activities that are associated with cell cycle transit. One of the earliest approaches used to monitor genome reduplication entailed scoring for the presence of cytological landmarks associated with nuclear division (ie, mitotic figures). An advantage of this approach is that no technical skills beyond the ability to perform routine tissue sectioning and histochemical staining are required. Moreover, the presence of mitotic figures unequivocally identifies nuclei that are dividing. Since the duration of M phase is markedly shorter than the other phases of the cell cycle, proliferation indices based on mitotic figures will be proportionately lower than those based on DNA synthesis assays (ie, S-phase assays). In light of this, analysis of cell populations with low levels of proliferation is somewhat problematic with this approach. This concern can be somewhat ameliorated by pretreating the experimental animal with a mitosis-blocking agent, such as colchicine. Finally, scoring mitotic figures cannot distinguish between cytokinesis and karyokinesis in cells that undergo multinucleation.

Direct assessment of DNA synthesis and/or content has been widely used as a marker for cellular proliferation. Several approaches have been used toward that end, including metabolic assays that monitor the incorporation of deoxyribonucleotide analogues into newly synthesized DNA as well as static assays that rely on microfluorometry or flow cytometry to quantify nuclear DNA content. Tritiated thymidine incorporation was one of the first metabolic-based methods used to monitor DNA synthesis in vivo. Experimental animals receive one or more injections of isotope, and after a short chase period, the tissue of interest is harvested and sectioned. The tissue sections are coated with photographic emulsion, and the autoradiogram is then developed after a suitable exposure period. Cells synthesizing DNA during the labeling period incorporate the radiolabeled nucleotide, which is evidenced by the presence of silver grains overlying their nuclei. Identification of these nuclei is quite unambiguous, provided that the autoradiographic background is reasonably low. This assay has the added advantages of being highly specific and relatively inexpensive and is semiquantitative to the extent to which nuclear silver grains can be accurately counted. Moreover, when used in a pulse-chase format, it is possible to determine whether the observed DNA synthesis ultimately culminates in cell division, multinucle-

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Selected Abbreviations and Acronyms

BrdU = bromodeoxyuridine
MHC = myosin heavy chain
nLAC = nuclear localized β-galactosidase
PCNA = proliferating cell nuclear antigen
X-GAL = 3-chloro-5-bromo-indolyl-β-galactopyranoside

ation, or polyploidization. Disadvantages of the approach include those that are inherent to the use of radioisotopes (which become particularly problematic when scaling up to larger animals), the cumbersome nature of autoradiographic emulsion application, and the time required for emulsion exposure.

The generation of monoclonal antibodies against the nucleotide analogue BrdU has facilitated the development of immune-based assays to monitor DNA synthesis. Experimental animals receive one or more injections of BrdU, which is incorporated into the nucleus of cells synthesizing DNA. After a brief chase period, the desired tissues are harvested and sectioned, and the cells synthesizing DNA during the labeling period are visualized by anti-BrdU immune histology. This approach has the advantage of being much more sensitive, rapid, and technically easier than assays based on thymidine incorporation. On the negative side, loss of tissue integrity resulting from the requisite permeabilization steps during sample processing may decrease the fidelity of cell identification. In addition, the great sensitivity of this immune-based assay increases the possibility of scoring false positives. For example, marked variation in nuclear immune reactivity is frequently observed between individual cells. In such instances, it is difficult to determine whether nuclei with comparatively weak signals should be scored as positive or negative for DNA synthesis. The use of BrdU for long-term (ie, pulse-chase) studies may also be problematic, because this analogue is known to affect proliferation and differentiation of cells in culture.

Direct quantification of nuclear DNA content has also been used to monitor DNA synthesis. Such analyses typically rely on microfluorometric analysis of Feulgen-stained nuclei and have the distinct advantage that ploidy can be accurately determined. As such, it is relatively easy to distinguish between de novo genomic replication versus repair DNA synthesis (a distinction that is potentially subjective with the metabolic assays and, in particular, with BrdU incorporation). Flow cytometric analysis of either intact cells or isolated nuclei prepared from the tissue of interest has also been used to monitor cell cycle progression in vivo. Both approaches suffer from the inherent disadvantage of being static assays. As such, it is difficult to determine whether elevated DNA levels reflect active DNA synthesis or, alternatively, cumulative changes in nuclear content. In light of this limitation, these approaches cannot accurately score samples with low levels of proliferation.

Our understanding of the molecular regulation of cell cycle progression has advanced dramatically over the past 10 years; as a result, many proteins that play an intimate role in cellular proliferation have been identified. It follows that monitoring the expression of these proteins would provide an alternative means by which to identify proliferating cells. Perhaps the most commonly used molecular marker for cellular proliferation is PCNA, which was initially identified as a component of the DNA replication fork. PCNA nuclear immune reactivity is widely accepted as a surrogate marker for DNA synthesis, and its use has the great advantage that the experimental subject requires no pretreatment. Consequently, PCNA analyses can readily be extended to larger species as well as to archived samples. Nonetheless, several points of caution should be raised regarding its use. Retrospective comparative studies of archival samples have demonstrated that the use of PCNA typically yields a higher proliferative index compared with thymidine or BrdU incorporation, a somewhat expected result since PCNA expression is not limited to the S phase of the cell cycle.[1–3] PCNA expression has also been detected in cells undergoing DNA repair[4] and apoptosis,[5] raising the possibility that not all PCNA-positive cells observed in a given tissue section are replicating. In addition, PCNA expression has been observed in nonproliferating cells bordering transplanted tumor cells, suggesting that the tumor may elaborate a paracrine factor(s) capable of inducing PCNA expression in the absence of DNA synthesis.[6]

Identification of Cardiomyocytes

Regardless of which marker is selected to monitor genome reduplication, assessment of cardiomyocyte DNA synthesis can only be as accurate as the assay used to distinguish cardiomyocyte nuclei from nonmyocyte nuclei. A priori, this would appear to be a trivial task. However, cardiomyocytes constitute only 20% to 30% of the total number of cells present in the adult heart, despite the fact that they constitute the majority of the organ mass. Consequently, the preponderance of nuclei present in histological sections of adult hearts are from noncardiomyocytes. Cardiomyocyte identification (and, more specifically, identification of cardiomyocyte nuclei) is thus of paramount importance, particularly at stages of development where the number of proliferating myocytes is small.

Light-microscopic analysis of histological sections has been the most common method used to identify cardiomyocytes in cell proliferation studies. Such analyses are commonly used in conjunction with metabolic-based DNA synthesis assays (tritiated thymidine or BrdU incorporation). Cardiomyocyte identification initially was based strictly on morphological criteria after histochemical staining. More recently, identification has been aided by the use of immunological reagents that recognize epitopes present in cardiomyocytes but not in other cardiac cell types (as, for example, sarcomeric MHC or actin). Cardiomyocyte identification can be further enhanced through the use of confocal laser scanning microscopy. The main advantage of confocal microscopy is that the effective fluorescence illumination can be restricted to a very narrow beam, essentially permitting optical sectioning as thin as 0.2-μm intervals. Consequently, section thickness does not negatively impact on the practical resolving power of the microscope, provided that individual optical sections are analyzed (as opposed to composite images composed of multiple optical sections).

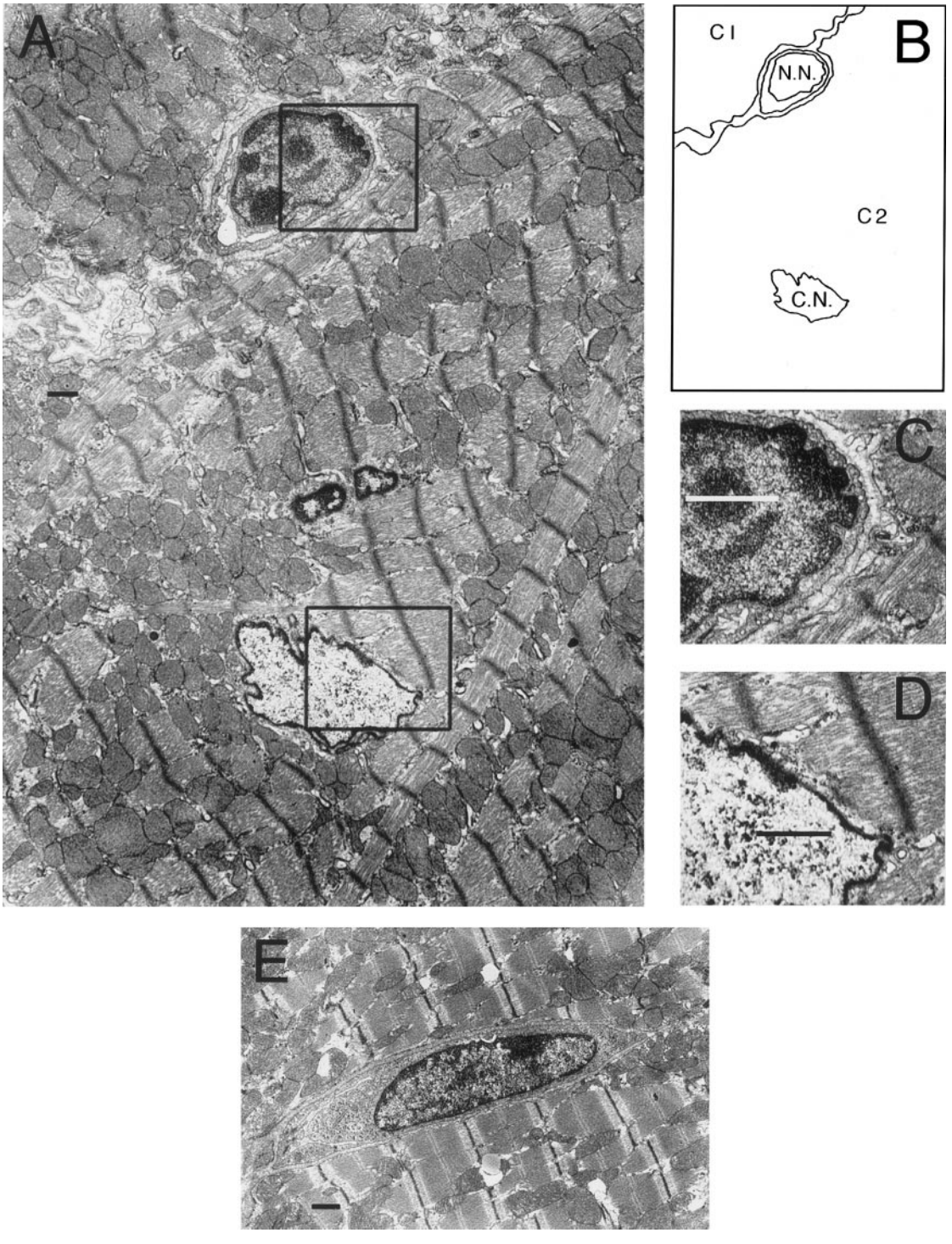

Figure 1. Ultrastructural analysis of the adult mouse myocardium. A, Low-magnification micrograph of a section prepared from an adult mouse heart. B, Schematic representation of the image shown in panel A. C1 and C2 refer to the 2 cardiomyocytes that are present in the image. C.N. refers to the nucleus present in cardiomyocyte C2. N.N. refers to a nonmyocyte nucleus. The positions of the cardiomyocyte and nonmyocyte plasma membranes are indicated. C, High-power view of the nonmyocyte nucleus (N.N.; the boxed region from panel A is shown). At this magnification, both the nonmyocyte and cardiomyocyte plasma membranes are discernible, and the extracellular space separating the 2 cells is apparent. D, High-power view of cardiomyocyte C2 nucleus (C.N.; the boxed region from panel A is shown). At this magnification, the rich myofiber content of the cytoplasm surrounding this nucleus is apparent. E, Additional example of a nonmyocyte that is tightly juxtaposed with cardiomyocytes. For all panels, bar=1 μm.

Although protocols that monitor expression of sarcomeric MHC or actin permit unambiguous identification of cardiomyocyte cytoplasmic structures (ie, myofibers), a certain degree of subjectivity remains when attempting to establish the identity of a given nucleus. This concern is best illustrated by examining images of cardiac muscle ultrastructure. Figure 1A shows a low-power electron micrograph of an adult mouse heart. Two cardiomyocytes and a nonmyocyte are

present in this field, as is depicted schematically in Figure 1B (C1 and C2 denote cardiomyocytes 1 and 2, respectively, whereas N.N. and C.N. denote a nonmyocyte nucleus and a cardiomyocyte nucleus, respectively). In this example, establishing the identity of the nonmyocyte nucleus required visualization of the plasma membranes, which are shown at higher magnification in Figure 1C. It is clear at this magnification that this nucleus does not reside in a cardiomyocyte. In contrast, high-magnification views of the cardiomyocyte nucleus clearly reveals that it resides in a myofiber-rich cytoplasm (Figure 1D) and, thus, is a cardiomyocyte nucleus. It would be impossible to make these designations using traditional light or confocal microscopy in the absence of a suitable plasma membrane stain. Such instances of ambiguous nuclear identification are not uncommon when light microscopy is used. Figure 1E shows an additional example of a nonmyocyte that is tightly juxtaposed to cardiomyocytes. Once again, designation of this nucleus as cardiomyocyte versus nonmyocyte would be quite subjective with traditional microscopic analyses.

Ultrastructural analysis provides an unequivocal means by which to distinguish cardiomyocyte and fibroblast nuclei, as is readily evident in the examples shown in Figure 1. When coupled with tritiated thymidine incorporation, ultrastructural analyses can provide indisputable evidence for cardiomyocyte DNA synthesis in vivo. Unfortunately, because sample preparation and screening is very labor intensive and because exposure times are great as a result of the requirement for ultrathin sections, the use of ultrastructural analyses for monitoring low rates of DNA synthesis (which is clearly the case for adult cardiomyocytes) is not practical. Monitoring mitotic figures using ultrastructural analyses is similarly problematic because of the low proliferation rates, particularly in light of the arguments regarding M-phase versus S-phase duration raised above.

The use of dispersed cell preparations provides an alternate approach to identify cardiomyocytes in DNA synthesis assays. Dispersed cell preparations from rodent hearts are typically obtained by retrograde collagenase perfusion on a Langendorff apparatus. Cardiomyocytes in the resulting dispersed cell preparation are readily distinguished from nonmyocytes by morphological criteria. DNA synthesis can be monitored by scoring for tritiated thymidine or BrdU incorporation or by PCNA staining. It is imperative that the cell preparations be well dispersed, because false positives might be scored if any fibroblasts remain attached to or associated with the cardiomyocytes. Although such interactions can frequently be identified by incorporating nuclear stains (DAPI, Hoechst) into the assay, this concern is nonetheless problematic because of the multinucleated nature of adult cardiomyocytes.

Given the issues regarding cardiomyocyte nuclear identification raised above, we are of the opinion that a certain degree of subjectivity exists when attempting to score adult cardiomyocyte DNA synthesis with traditional light, fluorescence, or confocal microscopy approaches. Although unequivocal cardiomyocyte nucleus identification is possible with either ultrastructural or dispersed cell analyses, their routine use for DNA synthesis assays is not practical in instances where the percentage of proliferating cells is low. Therefore, we sought to develop a rapid throughput assay that would eliminate the subjective aspects of cardiomyocyte nuclear identification. This approach relies on the use of a transgenic mouse model that expresses an nLAC reporter under the regulation of the α-cardiac MHC promoter.[7] These animals, designated MHC-nLAC, express the nLAC reporter in cardiac myocytes but not other cardiac cells. The cardiomyocyte nuclei are unambiguously identified by X-GAL staining of histological sections. DNA synthesis in this model can be scored by thymidine incorporation: the presence of silver grains over blue nuclei is indicative of cardiomyocyte DNA synthesis.

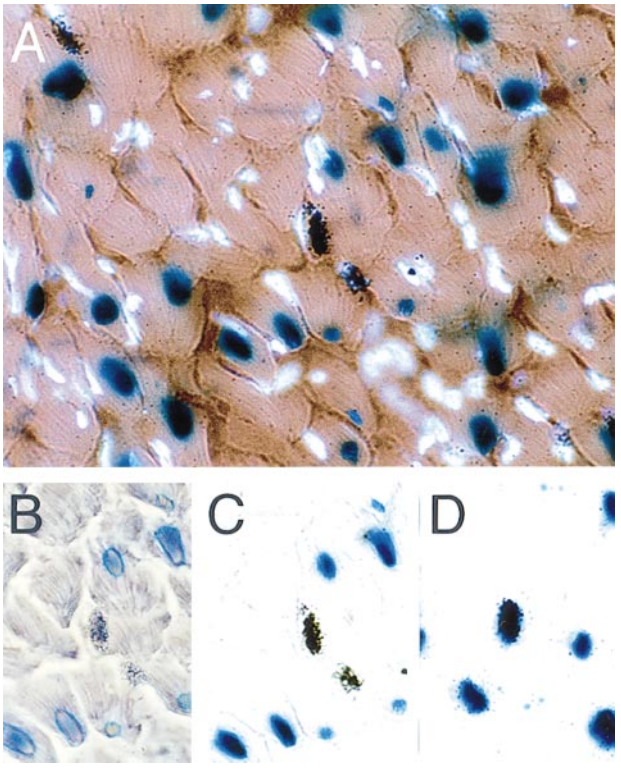

Figure 2. Use of the MHC-nLAC reporter gene assay to monitor cardiomyocyte DNA synthesis in adult mice. A, Photomicrograph of a section prepared from an MHC-nLAC mouse heart that received an injection of tritiated thymidine to monitor DNA synthesis. The section was stained with X-GAL and Hoechst before processing for autoradiography. The micrograph was taken under full fluorescence and partial bright-field illumination to permit simultaneous visualization of cardiomyocyte (intense dark blue) and noncardiomyocyte (pale light blue) nuclei. Silver grains are apparent over 2 nuclei in the center of the micrograph and over a third nucleus in the upper left corner of the micrograph. B, Micrograph of the central region of panel A (encompassing the 2 nuclei with silver grains) photographed under phase-contrast illumination. Note that one of the nuclei overlies a myofiber-rich cytoplasm. C, Micrograph of the same field depicted in panel B photographed under full bright-field illumination. Note that the nuclear silver grains do not colocalize with the intense blue X-GAL reaction product, indicating that the cells synthesizing DNA are not cardiomyocytes. D, Micrograph of a cardiomyocyte that was synthesizing DNA. Note the colocalization of silver grains and the intense blue X-GAL reaction product.

An example of this assay is depicted in Figure 2. Figure 2A shows a micrograph of a section prepared from the heart of an

MHC-nLAC mouse that received an injection of tritiated thymidine 2 hours before it was killed for study. The section was stained with X-GAL (to visualize the cardiomyocyte nuclei) and counterstained with Hoechst (to visualize noncardiomyocyte nuclei) before processing for autoradiography. The image shown in Figure 2A was photographed under simultaneous full fluorescence and partial bright-field illumination. The cardiomyocyte nuclei are readily identified by the intense blue X-GAL reaction product. It is also apparent that the nonmyocyte nuclei (which appear pale blue because of the fluorescent Hoechst staining) outnumber the cardiomyocyte nuclei. Two nuclei from cells synthesizing DNA are present in the middle of the field, as evidenced by the presence of overlying silver grains (the nucleus of a third cell that was synthesizing DNA is seen in the upper left corner of the micrograph). Figure 2B shows the region containing the 2 synthetic nuclei photographed under phase-contrast microscopy (the same field depicted in Figure 2A is shown: the image has been cropped). It is apparent that one of the synthetic nuclei overlies a myofiber-rich cytoplasm, as evidenced by the presence of cross striations around the entire periphery of the nucleus. Figure 2C shows the same field photographed under full bright-field illumination. The nucleus seen overlying the myofiber-rich cytoplasm in Figure 2B does not stain with X-GAL and, as such, is not a cardiomyocyte nucleus. This distinction would be difficult (if not impossible) to make via traditional light-microscopic analyses in the absence of the nuclear localized β-galactosidase marker. Cardiomyocyte DNA synthesis is also readily identified with this assay, as evidenced by the colocalization of silver grains and X-GAL staining (Figure 2D). This assay routinely yields a ventricular cardiomyocyte labeling index of $<0.0005\%$ in normal adult mouse hearts.

Although the MHC-nLAC assay provides a powerful means to identify cardiomyocyte nuclei, several potential caveats may result in an underestimate of cardiomyocyte DNA synthesis when using this model. These include issues related to the penetrance of transgene expression as well as the requirement that the MHC promoter remain transcriptionally during DNA synthesis. Transgene penetrance is easily checked by staining dispersed cell preparations from MHC-nLAC mice with X-GAL. Routine analysis of young adult mice (3 to 5 months) from our breeding colony consistently reveals that all of the cardiomyocytes identified by virtue of their rod-shaped morphology exhibit nLAC activity. Because essentially 100% of the cells expressed nLAC activity, transgene penetrance is not likely to be an issue with this model. The assay also requires that the MHC promoter remain transcriptionally active: silencing of the MHC promoter in cells synthesizing DNA would lead to an underestimate of the number of cycling cells present in a given experiment. Although this latter scenario is possible, it is unlikely, in light of the robust nuclear β-galactosidase activity that has been observed in the synthetic cells examined to date (for example, see Figure 2D). Furthermore, similar cardiomyocyte labeling indices were obtained in control experiments comparing the dispersed cell and MHC-nLAC assays.[8]

Studies Monitoring Cardiomyocyte DNA Synthesis During Normal and Pathological Myocardial Growth

Table 1 summarizes a series of studies that examined cardiomyocyte DNA synthesis in rats and mice during normal development. Because of space limitations, this list is not comprehensive: we apologize in advance to any individuals with similar data whom we have failed to cite here. The data are rank-ordered according to the age of the animals at the time of analysis. The chamber analyzed, percent synthetic cardiomyocytes, total number of cells screened, protocol used to monitor DNA synthesis, method used for cardiomyocyte identification, and reference citation for each entry are provided. A quick perusal of the data reveals that the reported values for cardiomyocyte DNA synthesis are in good agreement at early stages of heart development, where a relatively large percentage of the cells are proliferating. The main discrepancies appear in studies examining late neonatal and adult animals, where the overall level of cardiomyocyte DNA synthesis is markedly lower. Moreover, the fibroblast content of the heart is markedly increased at these stages of development. Table 2 summarizes a series of studies that examined cardiomyocyte DNA synthesis in response to myocardial pathology in rats and mice. The data are subdivided by species, and the injury model, chamber analyzed, percent synthetic cardiomyocytes, total number of cells screened, age of the animal, DNA synthesis assay, cardiomyocyte identification criteria, and reference citation are provided for each entry.

Summary and Conclusions

The data summarized here support the notion that cardiomyocyte DNA synthesis does occur in adult mammalian heart. The main discrepancy in the literature concerns the frequency at which this process occurs in healthy hearts and to what extent, if any, it is stimulated in diseased hearts. It is obvious that the ability to accurately measure these events is dependent on the fidelity of the method used to monitor DNA synthesis as well as the fidelity of the method used to identify cardiomyocytes. We have critiqued these methods, and the results summarized here should be interpreted in light of the relative strengths and weakness of the particular assay used. We are admittedly biased against those assays that we feel have a subjective component. Nonetheless, it is up to each investigator (and reader) to determine the extent to which the issues and concerns raised above may have affected the scoring of cardiomyocyte DNA synthesis in the studies cited.

Factors other than nuclear misidentification may have contributed to the different levels of cardiomyocyte DNA synthesis reported for adult hearts. We have focused most of our analyses on mice, largely because of the ease in altering cardiomyocyte gene expression via transgenesis in this species. Although we are quite confident that the rate of cardiomyocyte DNA synthesis in the normal adult mouse (as well as in the injury models that we have examined to date) is exceedingly low, it is possible that differences in the basal rates of adult cardiomyocyte DNA synthesis may exist between species. Resolution of this

TABLE 1. Compilation of Studies Examining Levels of Cardiomyocyte DNA Synthesis During Normal Development in Rats and Mice

Species	Age*	Chamber	Synthetic Cardiomyocytes, %	No. of Cells/Nuclei Screened†	DNA Synthesis Assay‡	Cardiomyocyte Identification	Reference
Rat	Fetal	LV	16.5	· · ·	BrdU	LM/I	Cheng et al[9] (1995)
Rat	18 ed	Vent	13.3	4000	3H (1)	LM/H	Marino et al[10] (1991)
Rat	0 d	Vent	5.8	4000	3H (1)	LM/H	Marino et al[10] (1991)
Rat	0 d	Vent	12	7200	3H (1)	LM/H	Clubb and Bishop[11] (1984)
Rat	1 d	LV	13	· · ·	BrdU	LM/I	Cheng et al[9] (1995)
Rat	1 d	Heart	1.98	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	2 d	Vent	8.3	· · ·	3H (1)	LM/H	Sasaki et al[13] (1970)
Rat	3 d	Vent	15	7200	3H (4)	LM/H	Clubb and Bishop[11] (1984)
Rat	4 d	Vent	10	400	BrdU	Isolated	Li et al[14] (1996)
Rat	5 d	LV	7.75	· · ·	BrdU	LM/I	Cheng et al[9] (1995)
Rat	5 d	Vent	3.9	4000	3H (1)	LM/H	Marino et al[10] (1991)
Rat	6 d	Vent	12	7200	3H (4)	LM/H	Clubb and Bishop[11] (1984)
Rat	7 d	Vent	4.2	· · ·	3H (1)	LM/H	Sasaki et al[13] (1970)
Rat	7 d	Heart	1.4	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	10 d	Vent	6	7200	3H (4)	LM/H	Clubb and Bishop[11] (1984)
Rat	11 d	LV	5.5	· · ·	BrdU	LM/I	Cheng et al[9] (1995)
Rat	14 d	Vent	1.5	4000	3H (1)	LM/H	Marino et al[10] (1991)
Rat	14 d	Vent	1.1	· · ·	3H (1)	LM/H	Sasaki et al[13] (1970)
Rat	14 d	Vent	3	7200	3H (4)	LM/H	Clubb and Bishop[11] (1984)
Rat	14 d	Heart	0.85	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	16 d	Vent	0	· · ·	PCNA	LM/H	Marino et al[15] (1996)
Rat	21 d	LV	0.5	· · ·	BrdU	LM/I	Cheng et al[9] (1995)
Rat	21 d	Vent	0.15	· · ·	3H (1)	LM/H	Sasaki et al[13] (1970)
Rat	21 d	Vent	1	7200	3H (4)	LM/H	Clubb and Bishop[11] (1984)
Rat	21 d	Heart	0.35	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	28 d	Vent	0.2	· · ·	3H (1)	LM/H	Nag et al[16] (1983)
Rat	28 d	Heart	0.1	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	30 d	LV	0.068	· · ·	3H (1)	LM/H	Wachtlova et al[17] (1977)
Rat	30 d	RV	0.076	· · ·	3H (1)	LM/H	Wachtlova et al[17] (1977)
Rat	30 d	LV	1.74	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	30 d	RV	1.96	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	30 d	LA	21.06	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	30 d	RA	18.54	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	38 d	LV	0.91	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	38 d	RV	1.14	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	38 d	LA	9.64	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	38 d	RA	10.31	· · ·	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	56 d	Vent	0.35	· · ·	BrdU	LM/I	Baba et al[19] (1996)
Rat	60 d	LV	0.2	· · ·	BrdU	LM/I	Cheng et al[9] (1995)
Rat	60 d	LV	0.16	21 000	BrdU	PH/N	Kajstura et al[20] (1994)
Rat	60 d	RV	0.13	21 000	BrdU	PH/N	Kajstura et al[20] (1994)
Rat	60 d	Heart	0	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	75 d	LV	0.25	9000	BrdU	PH/N	Reiss et al[21] (1993)
Rat	75 d	RV	0.15	9000	BrdU	PH/N	Reiss et al[21] (1993)
Rat	70–94+ d	Vent	0–0.004	· · ·	3H (1)	LM/H	Kuhn et al[22] (1974)

ed indicates embryonic day; LV, left ventricle; Vent, ventricles; RV, right ventricle; LA, left atrium; RA, right atrium; LM, light microscopy; I, immune histology; H, histochemistry; Isolated, dispersed cell analyses; PH, phase-contrast microscopy; N, nuclear staining; Marker, nLAC transgenic marker; MF, mitotic figures; and 3H, tritiated thymidine.

*Decimals indicate part of a day (eg, 0.5 d=12 h).

†Values are provided for those studies for which the total number of cells or nuclei screened could be determined.

‡Parenthetical numbers indicate the number of repeat injections.

TABLE 1. Continued

Species	Age*	Chamber	Synthetic Cardiomyocytes, %	No. of Cells/Nuclei Screened†	DNA Synthesis Assay‡	Cardiomyocyte Identification	Reference
Rat	90 d	Heart	0	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	Adult	Vent	0.45	4000	3H (1)	LM/H	Marino et al[10] (1991)
Rat	Adult	Vent	0	· · ·	PCNA	LM/H	Marino et al[15] (1996)
Rat	Adult	Vent	0.005	≈90 000	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	Adult	Atria	<0.02	≈90 000	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	Adult	Atria	<0.02	≈90 000	MF	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	Adult	Atria	2.6	3200	3H (10)	Isolated	Oberpriller et al[24] (1984)
Rat	Adult	Vent	0	· · ·	3H (1)	LM/H	Rumjantsev[25] (1970)
Rat	Adult	Vent	0.1	· · ·	3H (1)	LM/H	Morkin and Ashford[26] (1968)
Rat	Adult	Vent	0	· · ·	PCNA	LM/I	Reiss et al[27] (1994)
Rat	Adult	Vent	0	5000	PCNA	LM/H	Heron and Rakusan[28] (1995)
Rat	Adult	Heart	0	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	Adult	LV	0.39	2500	MC	Statistical	Anversa et al[29] (1991)
Rat	Adult	RV	1.37	2500	MC	Statistical	Anversa et al[29] (1991)
Rat	Adult	Heart	0	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	Adult	LV	1.91	2000	MC	Statistical	Anversa et al[29] (1991)
Rat	Adult	RV	1.85	2000	MC	Statistical	Anversa et al[29] (1991)
Rat	Adult	Heart	0	· · ·	MF	LM/H	Sasaki et al[12] (1968)
Rat	Adult	LV	2.46	2000	MC	Statistical	Anversa et al[29] (1991)
Rat	Adult	RV	3.15	2000	MC	Statistical	Anversa et al[29] (1991)
Mouse	10 ed	Vent	46.0	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	12 ed	Vent	37.0	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	12 ed	Vent	33.0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	12 ed	Atria	32.0	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	15 ed	Vent	26.3	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	15 ed	Atria	19.8	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	16 ed	Vent	32.3	1000	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	16 ed	Vent	20.1	303	3H (1)	EM	Erokhina and Rumyantsev[30] (1986)
Mouse	16 ed	Atria	20.7	159	3H (1)	EM	Erokhina and Rumyantsev[30] (1986)
Mouse	16 ed	Atria	19.5	1000	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	18 ed	Vent	12.1	1000	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	18 ed	Vent	19.1	141	3H (1)	EM	Erokhina and Rumyantsev[30] (1986)
Mouse	18 ed	Atria	15.6	206	3H (1)	EM	Erokhina and Rumyantsev[30] (1986)
Mouse	18 ed	Atria	12.8	1000	3H (1)	LM/H	Erokhina and Rumyantsev[30] (1986)
Mouse	18 ed	Vent	10.0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	0 d	Vent	6	30 000	3H (1)	LM/H	Nakagawa et al[35] (1988)
Mouse	0.04 d	Vent	2.3	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	0.58 d	Vent	2.3	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	0.96 d	Vent	3.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	1 d	LV	≈11	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	1.0 d	Vent	2.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	2 d	LV	≈12	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	2.1 d	Vent	3.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	2.29 d	Vent	6.0	400	BrdU	Isolated	Soonpaa et al[31] (1996)
Mouse	2–3 d	Vent	3.2	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	2–3 d	Atria	3.6	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	3 d	Vent	8	30 000	3H (1)	LM/H	Nakagawa et al[35] (1988)
Mouse	3 d	LV	≈12	· · ·	BrdU	LM/H	Machida et al[32] (1997)

TABLE 1. Continued

Species	Age*	Chamber	Synthetic Cardiomyocytes, %	No. of Cells/Nuclei Screened†	DNA Synthesis Assay‡	Cardiomyocyte Identification	Reference
Mouse	3.29 d	Vent	5.5	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	3.63 d	Vent	5.5	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	3–4 d	Vent	10	· · ·	3H (1)	Isolated	Brodsky et al[33] (1980)
Mouse	3–4 d	Vent	5.0	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	4.0 d	Vent	6.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	4.63 d	Vent	9.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	5 d	LV	≈12	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	6.33 d	Vent	7.5	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	7 d	Vent	7	30 000	BrdU	LM/H	Nakagawa et al[35] (1988)
Mouse	7 d	Vent	4.5	· · ·	3H (1)	Light	Erokhina and Rumyantsev[43] (1988)
Mouse	7 d	LV	≈12	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	7.0 d	Vent	5.5	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	7 d	Atria	4.8	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	7.2 d	Vent	5.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	8 d	Vent	9.4	2000	3H (1)	LM/H	Petersen and Baserga[34] (1965)
Mouse	10 d	LV	≈7	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	10 d	Vent	8.8	30 000	3H (1)	LM/H	Nakagawa et al[35] (1988)
Mouse	10 d	Vent	0.8	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	11 d	Atria	0.9	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	13 d	Vent	0.5	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	13 d	Atria	0.9	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	14 d	Vent	3.0	30 000	3H (1)	LM/H	Nakagawa et al[35] (1988)
Mouse	14 d	LV	≈2	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	15 d	Vent	0.04	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	15 d	Vent	0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	15 d	Atria	0.2	· · ·	3H (1)	LM/H	Erokhina and Rumyantsev[43] (1988)
Mouse	16 d	LV	≈2	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	16 d	Vent	6.0	2000	3H (1)	LM/H	Petersen and Baserga[34] (1965)
Mouse	16.17 d	Vent	0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	18 d	LV	≈2	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	20 d	Vent	0.2	30 000	3H (1)	LM/H	Nakagawa et al[35] (1988)
Mouse	20 d	LV	<1	· · ·	BrdU	LM/H	Machida et al[32] (1997)
Mouse	20 d	Vent	0.2	· · ·	3H (1)	LM/H	Nakagawa et al[35] (1988)
Mouse	20 d	Vent	0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	20.20 d	Vent	0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	32 d	Vent	2.2	2000	3H (1)	LM/H	Petersen and Baserga[34] (1965)
Mouse	Adult	Vent	0	6000	3H (1,7)	Isolated	Soonpaa and Field[36] (1994)
Mouse	Adult	Vent	0	400	3H (1)	Isolated	Soonpaa et al[31] (1996)
Mouse	Adult	Vent	0	20 000	3H (1)	Isolated	Soonpaa and Field[8] (1997)
Mouse	Adult	Vent	0.0004	270 000	3H (1)	Marker	Soonpaa et al[37] (1997)
Mouse	Adult	Vent	0.0006	180 000	3H (1)	Marker	Soonpaa and Field[8] (1997)
Mouse	Adult	Vent	<0.01	20 000	3H (1)	LM/H	Rumyantsev[38] (1966)
Mouse	Adult	Atria	0	18 000	3H (1)	Marker	Soonpaa and Field[8] (1997)
Mouse	150 d	Vent	0	2000	3H (1)	LM/H	Petersen and Baserga[34] (1965)
Mouse	100–200 d	Vent	0.04	90 000	3H (1)	LM/H	Nakagawa et al[35] (1988)

TABLE 2. Compilation of Studies Examining Levels of Cardiomyocyte DNA Synthesis During Pathophysiological Stimulus in Rats and Mice

Species	Injury Model	Chamber	Synthetic Cardiomyocytes, %	No. of Cell Nuclei Screened*	Age	DNA Synthesis Assay†	Cardiomyocyte Identification	Reference
Rat	Spark injury	Vent	3.5	4408	7 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Border	8.0	3262	7 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Vent	2.0	5247	14 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Border	3.2	2444	14 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Vent	0.6	3676	21 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Border	2.2	5535	21 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Vent	0.5	2501	28 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	Spark injury	Border	0.2	3071	28 d	3H (1)	LM/H	Nag et al[16] (1983)
Rat	LV infarct	LV	3.5	· · ·	30 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	RV	3.13	· · ·	30 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	LA	51.02	· · ·	30 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	RA	50.84	· · ·	30 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	LV	1.25	· · ·	38 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	RV	1.60	· · ·	38 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	LA	42.53	· · ·	38 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	LV infarct	RA	34.82	· · ·	38 d	3H (30)	LM/H	Rumyantsev and Borisov[18] (1987)
Rat	Renal hypertension	Vent	0.48	· · ·	56 d	BrdU	LM/I	Baba et al[19] (1996)
Rat	Renal hypertension	Vent	0.32	· · ·	6 mo	3H (1)	LM/H	Kuhn et al[22] (1974)
Rat	Coronary band	LV	1.17	9000	60 d	BrdU	PH/N	Kajstura et al[20] (1994)
Rat	Coronary band	RV	0.68	9000	60 d	BrdU	PH/N	Kajstura et al[20] (1994)
Rat	Coronary band	LV	2.3	9000	75 d	BrdU	PH/N	Reiss et al[21] (1993)
Rat	Coronary band	RV	0.9	9000	75 d	BrdU	PH/N	Reiss et al[21] (1993)
Rat	LV infarct	LA	3–4	· · ·	120–170 g	3H (1)	LM/H	Rumyantsev[39] (1974)
Rat	LV infarct	LA	10–30	· · ·	120–170 g	3H (3–5)	LM/H	Rumyantsev[39] (1974)
Rat	LV infarct	LA	43.6	1600	160 g	3H (10)	Isolated	Oberpriller et al[24] (1984)
Rat	LV infarct	RA	25.7	1600	160 g	3H (10)	Isolated	Oberpriller et al[24] (1984)
Rat	LV infarct 3 d post	LA	0.22	9000	150–200 g	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 5 d post	LA	3.85	15 000	150–200 g	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 8 d post	LA	1.45	6000	150–200 g	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LA infarct 14 d post	LA	0.70	13 500	150–200 g	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 40 d post	LA	0.79	6000	150–200 g	3H (1)	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 3 d post	LA	0.14	9000	150–200 g	MF	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 5 d post	LA	2.01	15 000	150–200 g	MF	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 8 d post	LA	0.15	6000	150–200 g	MF	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 14 d post	LA	0.37	13 500	150–200 g	MF	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	LV infarct 40 d post	LA	0.15	6000	150–200 g	MF	LM/H	Rumyantsev and Mirakjan[23] (1968)
Rat	Abdominal band	Vent	0	· · ·	200–220 g	3H (1)	LM/H	Morkin and Ashford[26] (1968)
Rat	Abdominal band	LA	1.36	· · ·	Adult	3H (1)	LM/H	Rumyantsev[40] (1983)
Rat	Abdominal band	LA	29.75	· · ·	Adult	3H (30)	LM/H	Rumyantsev[40] (1983)
Rat	Abdominal band	LA	1.44	· · ·	Adult	MF	LM/H	Rumyantsev[40] (1983)
Rat	Abdominal band	RA	1.32	· · ·	Adult	3H (1)	LM/H	Rumyantsev[40] (1983)
Rat	Abdominal band	RA	16.78	· · ·	Adult	3H (30)	LM/H	Rumyantsev[40] (1983)
Rat	Abdominal band	RA	0.29	· · ·	Adult	MF	LM/H	Rumyantsev[40] (1983)
Rat	Abdominal band	LV	0.217	· · ·	Adult	3H (30)	LM/H	Rumyantsev[40] (1983)

LV indicates left ventricle; RV, right ventricle; RA, right atrium; LA, left atrium; Vent, ventricles; LM, light microscopy; I, immune histology; H, histochemistry; Isolated, dispersed cell analyses; PH, phase-contrast microscopy; N, nuclear staining; Marker, nLAC transgenic marker; g, grams; MF, mitotic figures; and 3H, tritiated thymidine.

*Values are provided for those studies where the total number of cells or nuclei screened could be determined.

†Parenthetical numbers indicate the number of repeat injections.

TABLE 2. Continued

Species	Injury Model	Chamber	Synthetic Cardiomyocytes, %	No. of Cell Nuclei Screened*	Age	DNA Synthesis Assay†	Cardiomyocyte Identification	Reference
Rat	Renal hypertension	Heart	0.03–0.2	· · ·	NA	3H	LM/H	Sasaki et al[41] (1970)
Rat	Renal hypertension	Heart	0–0.1	· · ·	NA	MF	LM/H	Sasaki et al[41] (1970)
Rat	Abdominal band	Vent	0.0006	600 000	NA	3H (1)	LM/H	Meerson et al[42] (1968)
Mouse	Ispoproterenol	Vent	0	11 000	Adult	3H (1,7)	Isolated	Soonpaa and Field[36] (1994)
Mouse	Cautery	Vent	0	180 000	Adult	3H (1)	Marker	Soonpaa and Field[8] (1997)
Mouse	Cautery	Border	0.0083	36 000	Adult	3H (1)	Marker	Soonpaa and Field[8] (1997)
Mouse	Cautery	Atria	0.0055	18 000	Adult	3H (1)	Marker	Soonpaa and Field[8] (1997)
Mouse	Cautery	Vent	0	20 000	Adult	3H (1)	Isolated	Soonpaa and Field[8] (1997)
Mouse	Needle	Border	0.1–0.5	· · ·	Adult	3H (1)	LM/H	Rumyantsev[38] (1966)

issue will require that comparable analyses (as, for example, quantification of cardiomyocyte DNA synthesis with dispersed cell preparations or with a transgenic marker system) be performed for the species in question. Additionally, many of the early studies examining cardiomyocyte DNA synthesis in injured hearts used adolescent animals. This was particularly true for those studies documenting atrial cardiomyocyte DNA in response to cardiac injury. Given the data presented in Table 1, it is likely that developmental cardiomyocyte DNA synthesis was still active in these animals. Thus, it is not clear whether the cardiomyocyte labeling index reported in these studies resulted from an extension of the normal developmental program or alternatively from reactivation of DNA synthesis in a quiescent cell, a caveat that was explicitly raised by Oberpriller et al.[24] We have not seen enhanced atrial cardiomyocyte DNA synthesis in adult mice after cautery damage[8] or isoproterenol-induced hypertrophy (authors' unpublished data, 1998) using the MHC-nLAC model. Finally, the type and extent of myocardial injury may have an impact on the induction of cardiomyocyte DNA synthesis in adult hearts. Once again, this issue can be resolved only if comparable parameters (both the injury models and method of cardiomyocyte identification) are used for the species for which discrepancies have been noted.

An important issue that has not been addressed concerns the ultimate fate of adult cardiomyocytes that undergo DNA synthesis. We are not aware of any study that documents karyokinesis and/or cytokinesis in adult cardiomyocytes that have replicated their DNA. Indeed, it has been suggested that DNA synthesis in adult cardiomyocytes may presage apoptosis. In support of this, induction of adult cardiomyocyte DNA synthesis in vitro and in vivo with either adenoviral E1A or E2F1 engendered widespread apoptosis.[44–46] However, this does not appear to be an absolute relationship, because adult cardiomyocytes that synthesize DNA in response to D-type cyclin overexpression in transgenic mice are viable (Reference 37 and authors' unpublished data, 1998). It should be pointed out that these studies examined cardiomyocytes with experimentally altered gene expression. Although the fate of adult cardiomyocytes that synthesize DNA in the absence of genetic manipulation remains unknown, this issue could be addressed experimentally with pulse-chase experiments. It presently is not clear whether the cardiomyocyte DNA synthesis observed in adult hearts represents a normal physiological response or arises as a consequence of cumulative mutational changes (ie, a neoplastic response). The low frequency and random localization of synthetic cardiomyocytes in adult mouse hearts are more consistent with stochastic mutational events as opposed to a physiological response to global changes in the myocardium. Stochastic accumulation of mutations might relax cell cycle checkpoints such that DNA synthesis can occur. Given that adult cardiomyocytes are often multinucleated or are polyploid, only those mutations that activate proto-oncogenes or bestow dominant-negative properties to tumor suppressor genes would be detected. However, the pervasive DNA synthesis observed in long-term cultures of adult rat cardiomyocytes by Claycomb and Bradshaw[47] argues that, at least under these in vitro conditions, mutational activation is not a prerequisite for adult cardiomyocyte DNA synthesis. It is possible that manipulating the cardiomyocytes in this manner relaxes cell cycle checkpoints that would otherwise remain active in vivo. Alterations in the microenvironment in vivo may mimic these in vitro conditions and, in so doing, permit the activation of cardiomyocyte DNA synthesis in a limited number of cells. Importantly, cytokinesis has not been observed in long-term cultures of adult cardiomyocytes, in adult transgenic cardiomyocytes that synthesize DNA in response to targeted D-type cyclin expression,[37] or in response to adenovirus-mediated E1A or E2F gene transfer.[44–46] Thus, it would appear that cell cycle checkpoints downstream from S phase remain functional in all of these models.

It is important to unequivocally establish the rate of cardiomyocyte DNA synthesis in normal and diseased hearts, particularly in the human heart. From a therapeutic standpoint, intrinsic cardiomyocyte proliferation rates as low as 0.05% to 0.1% could, over the course of many months, result in a significant increase in cardiomyocyte number and, consequently, partial restoration of myocardial function in a diseased heart. Such an intrinsic growth rate, if it existed, could potentially be exploited. Con-

versely, if adult cardiomyocyte DNA synthesis ultimately leads to cell death and if this process occurs at a significant rate, the development of drugs blocking the process might enable physicians to salvage a considerable amount of myocardial function in diseased hearts. In either instance, if the number of cells synthesizing DNA is low, the therapeutic impact of such interventions would be negligible. Similar arguments can be made from an experimental standpoint. If a significant number of adult cardiomyocytes can reenter the cell cycle, it behooves the scientific community to identify those factors mediating the growth induction, because therapeutic administration of such factors might promote regenerative growth. If, on the other hand, the intrinsic rate of adult cardiomyocyte proliferation is exceedingly low, it is unlikely that such factors exist. As such, research efforts to identify such factors are futile.

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