elements exist. The discovery of new members of the STAT family, coupled with potential heterodimer formation, considerably broadens the possible range of specific STAT responses to different ligands. This ability of STAT proteins to interact both within the family and outside the family, as evidenced in IFN-α–stimulated gene factor 3 (ISGF-3) where Stat1α and Stat1β, Stat2, and the 48-kD DNA binding protein all interact (*23*), suggests a potential for forming and delivering transcription complexes to the nucleus specific to a wide array of different ligands.

REFERENCES AND NOTES

1. C. Schindler, X.-Y. Fu, T. Improta, R. Aebersold, J. E. Darnell Jr., *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7836 (1992).
2. X.-Y. Fu, C. Schindler, T. Improta, R. Aebersold, J. E. Darnell Jr., *ibid.*, p. 7840.
3. C. Schindler, K. Shuai, V. R. Prezioso, J. E. Darnell Jr., *Science* **257**, 809 (1992).
4. K. Shuai, C. Schindler, V. R. Prezioso, J. E. Darnell Jr., *ibid.* **258**, 1808 (1992).
5. K. Shuai, G. R. Stark, I. M. Kerr, J. E. Darnell Jr., *ibid.* **261**, 1744 (1993).
6. K. Shuai *et al.*, *Cell*, in press.
7. H. B. Sadowski, K. Shuai, J. E. Darnell Jr., M. Z. Gilman, *Science* **261**, 1739 (1993).
8. A. C. Larner *et al.*, *ibid.*, p. 1730.
9. O. Silvennoinen, C. Schindler, J. Schlessinger, D. E. Levy, *ibid.*, p. 1736.
10. S. Ruff-Jamison, K. Chen, S. Cohen, *ibid.*, p. 1733.
11. X.-Y. Fu and J.-J. Zhang, *Cell* **74**, 1135 (1993).
12. A. Bonni, D. A. Frank, C. Schindler, M. E. Greenberg, *Science* **262**, 1575 (1993).
13. H. Kotanides and N. C. Reich, *ibid.*, p. 1265.
14. Z. Zhong, Z. Wen, J. E. Darnell Jr., *Proc. Natl. Acad. Sci. U.S.A.*, in press.
15. K. Yamamoto *et al.*, unpublished results.
16. We searched for STAT family members in mouse cells and tissues because of the easy availability of differentiated cell material. With a pair of primers based on the DNA sequence of the SH2 domain of Stat1, we amplified and cloned an ~300-bp fragment of the mouse Stat1 cDNA and then selected the whole cDNA. Comparison of the entire human and mouse Stat1 sequences verified strong similarity of both amino acid (>95%) and nucleic acid sequences (~90%) throughout the entire coding region.
17. GenBank accession numbers for the sequences are U06922 for Stat3, U06923 for Stat4, and U06924 for mouse Stat1.
18. D. Lew, T. Decker, I. Strehlow, J. E. Darnell Jr., *Mol. Cell. Biol.* **11**, 182 (1991); R. N. Pearse, R. Feinman, K. Shuai, J. E. Darnell Jr., J. V. Ravetch, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4314 (1993).
19. B. J. Wagner, T. E. Hayes, C. J. Hoban, B. H. Cochran, *EMBO J.* **9**, 4477 (1990).
20. T. Akiyama *et al.*, *J. Biol. Chem.* **262**, 5592 (1987).
21. U. M. Wegenka, J. Buschman, C. Lutticken, P. C. Heinrich, F. Horn, *Mol. Cell. Biol.* **13**, 276 (1993).
22. C. Lütticken *et al.*, *Science* **263**, 89 (1994).
23. X.-Y. Fu, D. S. Kessler, S. A. Veals, D. E. Levy, J. E. Darnell Jr., *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8555 (1990).
24. F. M. Ausubel *et al.*, Eds., *Current Protocols In Molecular Biology* (Wiley, New York, 1993).

25. We thank X. Qin, W. Swiggard, and M. Nussenzweig for the mouse thymus library and W. Chen for the Rc/CMV vector. Supported by NIH grant AI32489 (J.E.D.).

21 December 1993; accepted 24 February 1994

Formation of Nascent Intercalated Disks Between Grafted Fetal Cardiomyocytes and Host Myocardium

Mark H. Soonpaa, Gou Young Koh, Michael G. Klug, Loren J. Field*

Fetal cardiomyocytes isolated from transgenic mice carrying a fusion gene of the α-cardiac myosin heavy chain promoter with a β-galactosidase reporter were examined for their ability to form stable intracardiac grafts. Embryonic day 15 transgenic cardiomyocytes delivered directly into the myocardium of syngeneic hosts formed stable grafts, as identified by nuclear β-galactosidase activity. Grafted cardiomyocytes were observed as long as 2 months after implantation, the latest date assayed. Intracardiac graft formation did not induce overtly negative effects on the host myocardium and was not associated with chronic immune rejection. Electron microscopy revealed the presence of nascent intercalated disks connecting the engrafted fetal cardiomyocytes and the host myocardium. These results suggest that intracardiac grafting might provide a useful approach for myocardial repair, provided that the grafted cells can contribute to myocardial function.

It is widely accepted that the adult mammalian myocardium has no regenerative capacity (*1*). As a consequence, cardiomyocyte loss as a result of injury or disease is irreversible. The ability to graft healthy myocytes into a diseased heart might compensate for myocardial loss, provided that the grafted cells are able to couple with host myocytes.

The feasibility of intracardiac grafting was first established with AT-1 cardiomyocytes, a differentiated tumor cell lineage derived from transgenic mice expressing the SV40 large T antigen (*2*). AT-1 cardiomyocytes formed stable grafts when introduced into the myocardium of syngeneic hosts, and graft formation did not overtly impair host heart function (*3*). However, no coupling was observed between grafted AT-1 cardiomyocytes (derived from the atria of transgenic mice) and host ventricular cardiomyocytes. Furthermore, because AT-1 cardiomyocytes are transformed, their ability to effect long-term myocardial repair is limited. Subsequent studies have assessed the capacity of skeletal myoblasts to form stable intracardiac grafts. C2C12 myoblasts delivered into the myocardium of syngeneic hosts differentiated to form grafts comprised of fused, multinucleated myotubes that were stable for at least 6 months (*4*). Furthermore, graft myocytes stopped cell cycling by 1 month after implantation, as measured by [^{3}H]thymidine incorporation. Surface electrocardiographic and plasma enzyme analyses did not detect any overtly negative effects on the host heart. However, it was not clear if the grafted C2C12 myocytes were able to couple to host cardiomyocytes, a prerequisite for this approach to successfully repair a diseased heart.

To explore further the use of intracardiac grafting as a means to effect myocardial repair, we generated transgenic mice that carry a fusion gene comprised of the α-cardiac myosin heavy chain (MHC) promoter and a modified β-galactosidase (β-gal) reporter (nLAC) (*5*). The nLAC reporter carries the SV40 nuclear transport signal, which results in the accumulation of β-gal activity in the nucleus of targeted cells. Four transgenic lineages were produced, and two (designated MHC-nLAC-2 and MHC-nLAC-4) were selected for further analyses. To ensure that the MHC-nLAC transgene would provide a suitable cell lineage marker, we assessed β-gal activity in transgenic cardiomyocytes. Single cell preparations generated by retrograde collagenase perfusion (*6*) were examined simultaneously for β-gal activity and 4,6-diamino-2-phenylindole (DAPI) epifluorescence (Fig. 1, A and B). Almost all (99.0 ± 0.45%, n = 400 for MHC-nLAC-2 and 100.0 ± 0.00%, n = 200 for MHC-nLAC-4) of the transgenic cardiomyocyte nuclei expressed β-gal, whereas no β-gal activity was detected in noncardiomyocytes. In addition, no nuclear β-gal activity was detected in nontransgenic control cardiomyocytes.

Single cell suspensions were prepared by collagenase digestion of hearts harvested from embryonic day 15 MHC-nLAC-2 or MHC-nLAC-4 transgenic mice (*7*). Greater than 95% of the cardiomyocytes isolated by this technique were viable, as evidenced by dye exclusion assay. Cardiomyocytes were delivered to the left ventricular free wall of syngeneic nontransgenic animals (*8*). Grafted cardiomyocytes were readily and unambiguously identified by virtue of the nuclear β-gal activity encoded by the MHC-nLAC transgene (Fig. 1, C and D). Grafted cardiomyocytes

Krannert Institute of Cardiology, Indiana University School of Medicine, 1111 West 10th Street, Indianapolis, IN 46202–4800, USA.

*To whom correspondence should be addressed.

were frequently observed at sites distal to the point of delivery; it presently is not clear whether this distribution of grafted cells reflects cardiomyocyte migration or simple spreading of the cells along a tissue plane. Approximately 58% (11/19) of the animals receiving intracardiac injections of fetal cardiomyocytes developed grafts. This frequency of successful graft formation is likely to increase as cell preparation and implantation protocols are optimized.

Light microscopy of hematoxylin and eosin (H and E) stained sections processed for β-gal activity indicated that grafted cardiomyocytes (blue nuclei) were juxtaposed directly with host cardiomyocytes (purple nuclei, Fig. 1E). The hearts depicted in Fig. 1 were harvested at 19 days after grafting; as such, the engrafted cells correspond to those of a 13-day-old mouse, because the fetal cells were obtained from 15-day-old embryos and mouse gestation is 20 to 21 days. At this stage of development, the mouse heart has not completed the normal program of hypertrophic growth. Consequently, the nucleus to cytoplasm ratio is somewhat greater in the engrafted cells. No differences in the nucleus to cytoplasm ratio were observed between graft and host cardiomyocytes at 2 months after grafting (9); in this case the donor cardiomyocytes had sufficient time to complete the normal program of hypertrophic growth. Additional H and E analyses indicated that the fetal grafts were not encapsulated by fibrous scar tissue (Fig. 1, E and F). The observed proximity of graft and host cardiomyocytes and absence of encapsulation are prerequisites for coupling between the two cell types. Of interest, grafted cardiomyocytes appeared to align themselves in circumferential arrays parallel to the host myocardium. A similar phenomenon was observed with C2C12 myoblast grafts (*4*).

Consecutive sections of a 19-day-old intracardiac graft were processed for staining with H and E, β-gal activity, and macrophage and leukocyte immunoreactivity (Fig. 1, F to I, respectively). Although no evidence for macrophage, monocyte, lymphocyte, natural killer cell, or leukocyte infiltration was detected, we cannot rule out the possibility that some of the high nucleus to cytoplasm ratio observed in Fig. 1, E and F, results from an inflammatory response. As a positive control for the immunohistology, grafts of incompatible major histocompatability complex haplotype were produced (Fig. 1, J and K); graft rejection was clearly evident in these hearts. [^{3}H]thymidine uptake indicated that only 0.6% (n = 156) of the β-gal–positive nuclei were synthesizing DNA, although noncardiomyocyte DNA synthesis was apparent (Fig. 1, L and M). Because the embryonic day 15 donor cells were still mitotically active when grafted (labeling index of ~29%, n = 400) (*1*, *10*), the exceedingly low level of DNA synthesis observed in β-gal–positive cells at 19 days after grafting suggests that the MHC-nLAC fetal cardiomyocytes had undergone terminal differentiation. This notion is further supported by the activity of the MHC-nLAC transgene in the grafted cells; αMHC is the classic contractile protein isoform associated with ventricular cardiomyocyte terminal differentiation in rodents.

The juxtaposition of graft and host cardiomyocytes observed by light microscopy prompted us to determine if direct intercellular coupling could be detected between the two cell types. The X-gal reaction

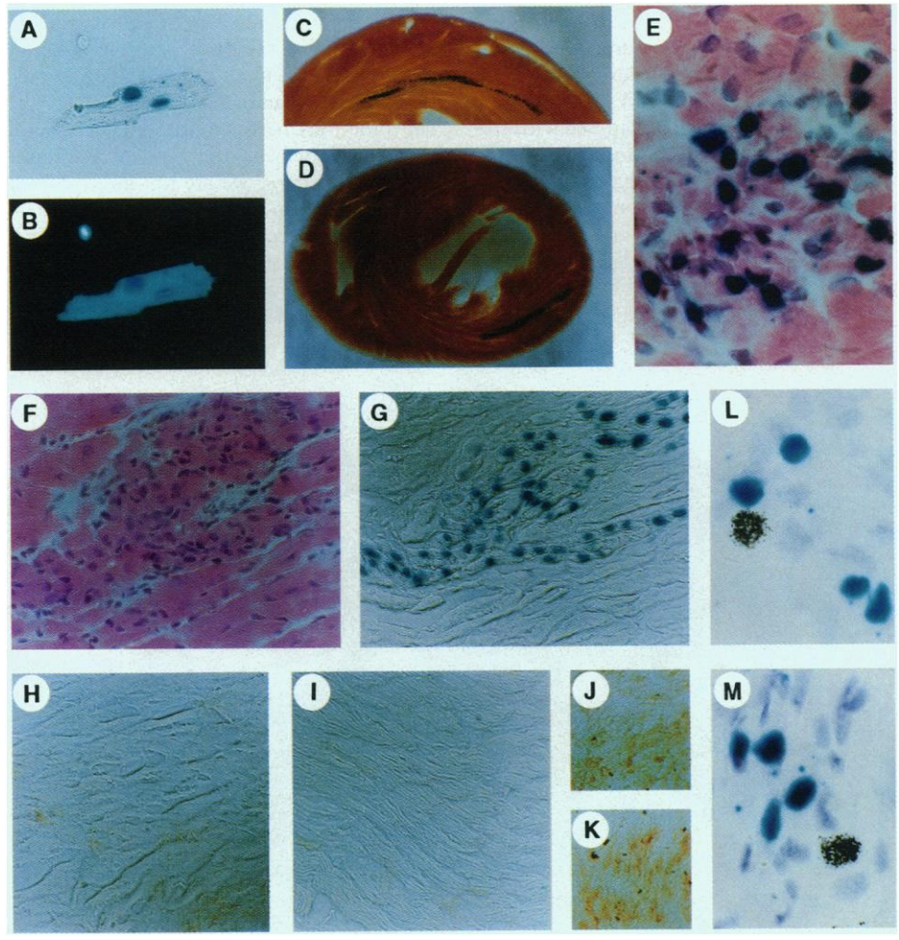

Fig. 1. Histologic analysis of MHC-nLAC cardiomyocytes and fetal cardiomyocyte grafts. (**A**) Single cell preparation of MHC-nLAC cardiomyocytes stained for β-gal activity (magnification, ×400). (**B**) Epifluorescent photomicrograph of field shown in (A), stained with DAPI (magnification, ×400). (**C** and **D**) Survey photomicrographs of coronal sections of hearts that carry MHC-nLAC fetal cardiomyocyte grafts, stained with X-gal. (**E**) High-power photomicrograph of MHC-nLAC intracardiac graft, H and E plus β-gal activity stain (magnification, ×1000). (**F** to **I**) Consecutive sections from an MHC-nLAC intracardiac graft showing staining for H and E, β-gal activity, and macrophage and leukocyte infiltration, respectively (magnification, ×400). (**J** and **K**) Positive controls for antibody to macrophage and antibody to leukocyte immunostaining, respectively (magnification, ×400). (**L** and **M**) Assessment of DNA synthesis in MHC-nLAC graft; sections were stained for β-gal activity and then processed for autoradiography. For H and E, X-gal, immunohistology, and thymidine analyses, hearts were cryoprotected, embedded, and sectioned at 10 μm as described (*16*). Sections post-fixed in acetone:methanol (1:1) were stained with H and E according to the manufacturer's specifications (Sigma). For β-gal assay, sections were post-fixed in acetone:methanol (1:1) and overlaid with X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) (1 mg/ml), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 2 mM magnesium chloride in phosphate-buffered saline (PBS). For immunohistology, antibodies against the common leukocyte antigen CD25 and macrophage Mac-1 antigen CD11b/18 (M1/9.3HL and M1/70HL; Boehringer Mannheim, Indianapolis, Indiana) were used; signal was visualized by an avidin-biotin (ABC) kit (Vector Labs, Burlingame, California). The antibody to macrophage cross-reacts with monocytes, lymphocytes, and natural killer cells. [^{3}H]thymidine incorporation methods were as previously described (*3*, *4*). For visualization of nuclei in single cell preparations, slides were stained with DAPI in PBS (0.28 μM, Boehringer Mannheim), washed in PBS, and wet-mounted in 2% n-propyl gallate dissolved in glycerol. Methods for the coronal heart sections were as described (*16*).

product is an electron-dense precipitate, which can be detected by transmission electron microscopy (TEM) (*11*). Vibratome sections from glutaraldehyde perfusion–fixed hearts were stained for β-gal activity (*12*), and grafted regions thus identified were trimmed and processed for TEM. Light microscopy of 1-μm sections revealed that the X-gal reaction product had a perinuclear appearance owing to a slight degree of nuclear leaching that occurred during the embedding process (*9*). Analysis of consecutive thin sections by TEM revealed host cardiomyocytes directly juxtaposed with the grafted cells (Fig. 2A). Host and donor cardiomyocytes were readily distinguished by the absence or presence, respectively, of perinuclear X-gal reaction product, which appeared as a crystalloid deposit at high magnification (Fig. 2, B and D). Intercalated disks were present between the host and graft cardiomyocytes (Fig. 2C), suggesting a high degree of intercellular coupling. Many examples of such coupling between host and graft cardiomyocytes were observed throughout the grafted regions. Importantly, intercellular connections could be traced from β-gal–positive cardiomyocytes through numerous host cells, thus demonstrating that grafted cardiomyocytes could be participating in a functional syncytium. Unfortunately, the processing required to obtain adequate nuclear X-gal staining prohibited the unambiguous identification of gap junctions between the engrafted fetal cardiomyocytes and the host myocardium. However, because we are not aware of any instances in which the formation of intercalated disks occurs in the absence of gap junctions, it is quite likely that the grafted cells are in fact electrically coupled. Definitive proof of electrical coupling is dependent on physiologic analyses that monitor either dye or action potential propagation between host and graft cardiomyocytes.

In addition to documenting the presence of abundant intercellular coupling between grafted and host cardiomyocytes, the TEM analyses revealed that the grafted cardiomyocytes were highly differentiated. Normal characteristics of adult cardiomyocytes were observed, including myofibrillae forming complete sarcomeres, numerous junctional complexes between cells, and abundant mitochondria. Indeed, aside from the presence of the X-gal reaction product, grafted cardiomyocytes were indistinguishable from host cells. Further, binucleated, β-gal–positive cells could be detected in the intracardiac grafts (*9*). Because binucleation is a characteristic of adult rodent cardiomyocytes (*1, 10*), this observation further supports the notion that the grafted cardiomyocytes have undergone terminal differentiation. Finally, surface electrocardiogram (ECG) recordings were used to determine if the presence of coupled fetal cardiomyocyte grafts negatively influenced host heart rhythm (Fig. 3). ECG traces from graft-bearing animals were indistinguishable from those of sham-operated controls and exhibited P waves and QRS complexes

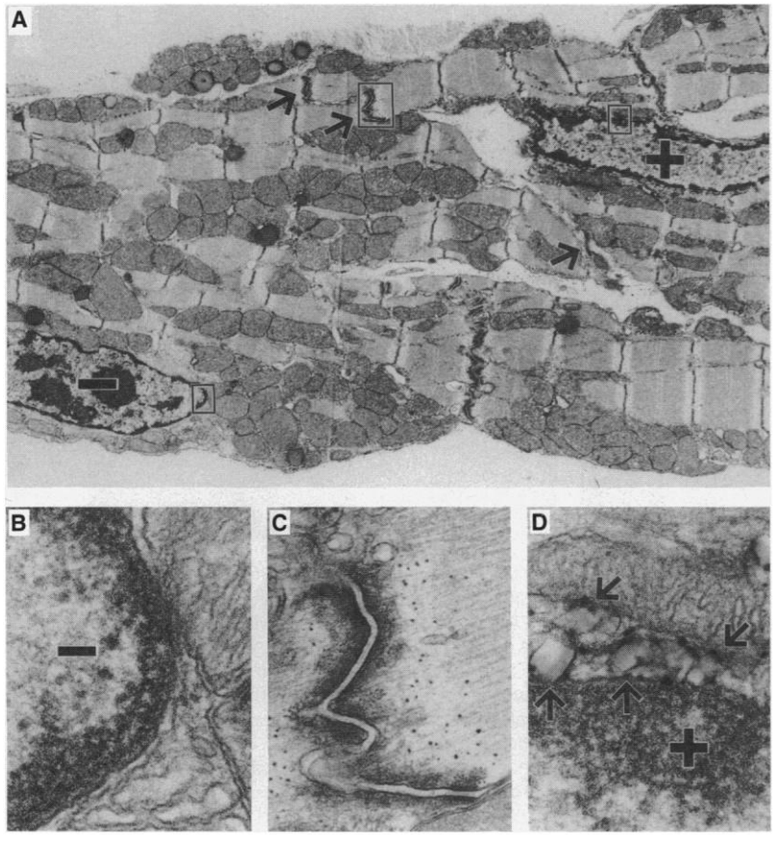

Fig. 2. Transmission electron microscopy analysis of fetal cardiomyocyte grafts. (**A**) Transmission electron microscopy of a host cardiomyocyte juxtaposed to an engrafted fetal cardiomyocyte. (−) indicates the nucleus of the host cardiomyocyte and (+) indicates the nucleus of the donor MHC-nLAC cardiomyocyte. Arrows indicate an intercalated disk that connects the two cells. Enlargements of the blocked regions are depicted in (B) to (D). (**B**) High-power view of the host cardiomyocyte nucleus shown in (A); note the complete absence of crystalloid X-gal reaction product in the perinuclear region; (−), nucleus. (**C**) High-power view of a region of the intercalated disk [blocked in (A)] that connects the host and fetal cardiomyocytes. (**D**) High-power view of the MHC-nLAC fetal cardiomyocyte nucleus shown in (A); note the presence of crystalloid X-gal reaction product (arrows) in the perinuclear region. (+); nucleus. MHC-nLAC fetal grafts were localized in coronal heart sections as described (*12*). All TEM protocols were as previously described (*3, 4*).

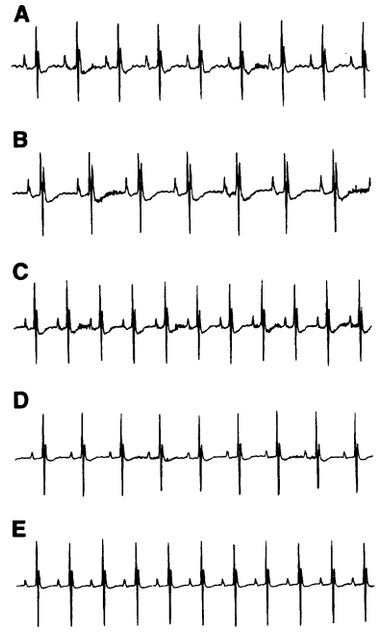

Fig. 3. ECG analysis of hearts bearing fetal cardiomyocyte grafts. (**A**) ECG trace from a sham operation. (**B** to **E**) ECG traces from animals with MHC-nLAC fetal cardiomyocyte grafts. Mice were anesthetized (2.5% Avertin, 0.015 ml per gram of body weight, intraperitoneally; Fluka Chemicals, Ronkomkoma, New York), surface electrodes were placed in the standard lead 1 position, and ECGs were recorded with a Narco Biosystems (Houston, Texas) high-gain amplifier coupled to an A/D converter (Coulbourn Instruments, Lehigh Valley, Pennsylvania).

(which correspond to atrial and ventricular depolarization, respectively) typical for mice. There was no evidence for cardiac arrhythmia in graft-bearing animals, despite the apparent presence of a high degree of intercellular coupling between grafted and host cardiomyocytes.

The donor transgenic cardiomyocytes used in this study were mitotically active when grafted. Although it is presently not clear if cell cycling is a prerequisite for successful graft formation, the time course of [^{3}H]thymidine incorporation and the use of donor transgenic cardiomyocytes from different stages of development should address this issue. If cardiomyocyte cycling is in fact required for graft formation, the ability to transiently induce proliferation in vitro could mean that biopsied adult cardiomyocytes could be used for autologous grafting experiments. Recent studies using transformed cardiomyocytes derived from transgenic mice have identified three endogenous proteins that form stable complexes with SV40 large T antigen (*13*). By analogy to skeletal muscle (*14*), these proteins may mediate cardiomyocyte terminal differentiation and consequently represent potential targets (either independently or in combination) with which to engender transient cardiomyocyte proliferation in vitro. In addition to fetal hearts, cardiomyocytes derived from embryonic stem cells (*15*) may be a viable source for donor cardiomyocytes. Intracardiac grafting of such cells might be useful for myocardial repair, provided that the grafted cells can contribute to myocardial function.

REFERENCES AND NOTES

1. P. P. Rumyantsev, in *Growth and Hyperplasia of Cardiac Muscle Cells*, B. M. Carlson, Ed. (Harwood, New York, 1991), pp. 3–68.
2. L. J. Field, *Science* **239**, 1029 (1988); M. E. Steinhelper *et al.*, *Am. J. Physiol.* **259**, H1826 (1990); J. B. Delcarpio, N. A. Lanson Jr., L. J. Field, W. C. Claycomb, *Circ. Res.* **69**, 1591 (1991); N. A. Lanson Jr., C. C. Glembotski, M. E. Steinhelper, L. J. Field, W. C. Claycomb, *Circulation* **85**, 1835 (1992).
3. G. Y. Koh, M. H. Soonpaa, M. G. Klug, L. J. Field, *Am. J. Physiol.* **264**, H1727 (1993).
4. G. Y. Koh, M. G. Klug, M. H. Soonpaa, L. J. Field, *J. Clin. Invest.* **92**, 1548 (1993).
5. The MHC promoter consisted of 4.5 kb of 5′ flanking sequence and 1 kb of the gene encompassing exons 1 through 3 up to but not including the initiation codon [J. Gulick, A. Subramaniam, J. Neumann, J. Robbins, *J. Biol. Chem.* **266**, 9180 (1991); A. Subramaniam *et al.*, *ibid.*, p. 24613]. The nLAC reporter was modified to carry both a eukaryotic translation initiation site and the SV40 nuclear localization signal [E. H. Mercer, G. W. Hoyle, R. P. Kapur, R. L. Brinster, R. D. Palmiter, *Neuron* **7**, 703 (1991)]. The transgene also carried an intron, as well as transcriptional termination and polyadenylation signals from the mouse protamine 1 gene. For generation of transgenic mice, MHC-nLAC insert DNA was purified by adsorption onto glass beads, dissolved at a concentration of 5 μg/ml, and microinjected into the nuclei of one-cell inbred C3Heb/FeJ embryos according to established protocols [B. Hogan, F. Costantini, E. Lacy, in *Manipulating the Mouse Embryo—A Laboratory Manual*, F. Constantini and E. Lacy, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986)]. Analysis by polymerase chain reaction (PCR) was used to identify founder animals and to monitor transgene segregation. The sense strand primer 5′-GGTGGGGGCTCTTCAC-CCCCAGACCTCTCC-3′ was localized to the MHC promoter, and the antisense strand primer 5′-GCCAGGGTTTTCCCAGTCACGACGTTGT-3′ was localized to the nLAC reporter. PCR analyses were as described [M. E. Steinhelper, K. L. Cochrane, L. J. Field, *Hypertension* **16**, 301 (1990)].
6. Transgenic animals were heparinized (10,000 U/kg, intraperitoneally) before they were killed by cervical dislocation. Hearts were placed in a beaker of gassed (95% O_2, 5% CO_2) KHB buffer (105 mM NaCl, 20 mM $NaHCO_3$, 3.8 mM KCl, 1 mM KH_2PO_4, 1.2 mM $MgSO_4$, 0.01 mM $CaCl_2$, 1 mM mannitol, 10 mM taurine, 10 mM dextrose, and 5 mM sodium pyruvate). Hearts were then hung by the aorta and perfused with gassed KHB (0.5 ml/min at 37°C) containing 2.5 mM EGTA for 5 min, followed by 0.17% collagenase (type I; Worthington Biochemical, Freehold, NJ) in KHB. Hearts were perfused until flaccid and the ventricles were minced with scissors, and isolated cells were obtained by trituration with a Pasteur pipette. After at least 1 hour of formalin fixation, suspensions were filtered and smeared onto positively charged slides (Superfrost Plus, Fisher, Pittsburgh, PA) and allowed to dry.
7. For isolation of single cells for injection, females with 15-day-old embryos (onset of pregnancy determined by vaginal plugs) were killed by cervical dislocation. Embryos were removed and decapitated, hearts were harvested under PBS, and ventricles and atria were separated. Transgenic ventricles (identified by cardiac β-gal activity) were digested in 0.1% collagenase (Worthington) in DPBS (Dulbecco's phosphate-buffered saline; Sigma) for 45 min and were triturated with a Pasteur pipette in PC-1 medium (Ventrex, Coons Rapids, MN) with 10% fetal bovine serum, resulting in a suspension of single cells.
8. Immediately after isolation, fetal cardiomyocytes were washed three times with DPBS and injected directly into the ventricular myocardium of syngeneic mice (Jackson Laboratory, Bar Harbor, ME) under open-heart surgery as described [H. A. Rockman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8277 (1991)]. Cells (1×10^4 to 10×10^4) were injected in a volume of 2 to 3 μl with the use of a plastic syringe fitted with a 30-gauge needle.
9. M. H. Soonpaa, G. Y. Koh, M. G. Klug, L. J. Field, data not shown.
10. M. H. Soonpaa, unpublished observations; W. Y. Brodsky, A. M. Arefyeva, I. V. Uryvaeva, *Cell Tissue Res.* **210**, 133 (1980); F. J. Clubb Jr. and S. P. Bishop, *Lab. Invest.* **50**, 571 (1984).
11. A. D. Loewy, P. C. Bridgman, T. C. Mettenleiter, *Brain Res.* **555**, 346 (1991); T. Mikawa, L. Cohen-Gould, D. A. Fischman, *Dev. Dyn.* **195**, 133 (1992).
12. For coronal sections, mice were killed by cervical dislocation and hearts were harvested and perfused on a Langendorff apparatus with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). After immersion fixation overnight in the same buffer, 200-μm coronal sections were made with a vibratome (Campden, London, United Kingdom). To localize the graft, we stained sections for β-gal activity with X-gal as described above.
13. A. I. Daud, N. A. Lanson Jr., W. C. Claycomb, L. J. Field, *Am. J. Physiol.* **264**, H1693 (1993).
14. W. Gu *et al.*, *Cell* **72**, 309 (1993); T. Endo and S. Goto, *J. Biochem.* **112**, 427 (1992).
15. T. C. Doetschman, H. Eistetter, M. Katz, W. Schmidt, R. Kemler, *J. Embryol. Exp. Morphol.* **87**, 27 (1985).
16. G. R. Bullock and P. Petrusz, in *Techniques in Immunocytochemistry* (Academic Press, New York, 1983), vol. 1, pp. 188–190.

17. We thank H. Wang, D. Field, and S. H. Koh for technical assistance, J. Robbins (University of Cincinnati School of Medicine) for the mouse α-cardiac MHC promoter, and J. Peschon (Immunex Corporation) for the nLAC reporter. Supported by National Heart, Lung, and Blood Institute grant HL45453. This work was done during the tenure of an Established Investigatorship from the American Heart Association (L.J.F.). M.G.K. was supported by a predoctoral fellowship from the American Heart Association, Indiana affiliate. G.Y.K. is the recipient of a Research Grant-in-Aid from the American Heart Association, Indiana affiliate.

14 October 1993; accepted 21 January 1994

Characterization of Type I Receptors for Transforming Growth Factor–β and Activin

Peter ten Dijke, Hidetoshi Yamashita, Hidenori Ichijo,* Petra Franzén, Marikki Laiho, Kohei Miyazono, Carl-Henrik Heldin†

Transforming growth factor–β (TGF-β) and activin exert their effects by binding to heteromeric complexes of type I and type II receptors. The type II receptors for TGF-β and activin are transmembrane serine-threonine kinases; a series of related receptors, denoted activin receptor–like kinase (ALK) 1 to 5, have recently been identified, and ALK-6 is described here. ALK-5 has been shown to be a functional TGF-β type I receptor. A systematic analysis revealed that most ALKs formed heteromeric complexes with the type II receptors for TGF-β and activin after overexpression in COS cells; however, among the six ALKs, only ALK-5 was a functional TGF-β type I receptor for activation of plasminogen activator inhibitor–1, and only ALK-2 and ALK-4 bound activin with high affinity.

The TGF-β proteins belong to a family of dimeric proteins that regulate the growth, differentiation, and metabolism of many cell types (*1*), and they are members of a larger superfamily of structurally related proteins that includes activins, inhibins, bone morphogenetic proteins, and Müllerian inhibiting substance. Both TGF-βs and activins exert their effects through binding to specific cell surface receptors (*2*). The TGF-β type I (53 kD) and type II (75 kD) receptors are indispensable for signal transduction and form heteromeric complexes on ligand binding (*3*, *4*). The