Cell-to-Cell Coupling Between Host and Donor Cells in the In Situ Myocardium

André G. Kléber

Atrial and ventricular working myocardium consists of individual myocytes. Normal electrical and probably metabolic function implicates the presence of connexin (Cx) proteins that ensure intercellular current flow during cardiac electrical excitation. The biophysical properties, the molecular structure, and the responsiveness of connexins to metabolites, drugs, and ions have been relatively well characterized. Both diffusion experiments with fluorescent molecules of varying size and double-voltage clamp experiments have shown that the pores of connexins are relatively large and allow diffusion of molecules up to a size of approximately 1000 M₉. Connexin channels present interesting and unique features that are distinct from ion channels embedded in the cell surface membrane. Each connexin channel is formed by the docking of 2 juxtaposed connexons synthesized by the adjacent cells, and each connexon is formed by 6 connexin proteins. In heart, 3 types of connexins play a major role in different regions, Cx43, Cx40, and Cx45 (eg.3,4). Double-immunohistochemical staining has shown that connexins are often colocalized in gap junctions. This colocalization may reflect heterotypic and/or heteromeric connexon formation. In vitro, such formation has shown to produce a multitude of electric conductance states (eg.5), but the exact role of mixed channels in vivo remains to be clarified.

In this issue of Circulation Research, Rubart et al demonstrate in an elegant way that embryonic cardiomyocytes (embryonic day 13), when injected into the left ventricle of adult mice of the same genetic background, fully integrate into the tissue matrix of the adult host cells and form gap junctions showing a positive signal for Cx43. Although an ultimate proof for electrical cell-to-cell coupling is not provided (this is extremely difficult if not impossible to achieve in vivo with currently known methods), the authors show normal Ca²⁺ transients in the donor cells and synchronization of Ca²⁺ transients between donor and host cells. This indicates that the integrated donor cells can electrically communicate with their host neighbors and contribute to the contractile function. The findings of Rubart et al are certainly relevant for the understanding of the effect of cardiomyocyte transplantation into a diseased heart. Such transplantation has been shown to induce formation of new blood vessels and produce functional benefit; however, the question of functional integration has not been answered. The findings of Rubart et al are related to some basic questions of gap junction biology: (1) In what conditions do we observe de novo formation of gap junctions between adult cardiomyocytes and between adult and embryonic or neonatal cardiomyocytes? (2) What is the time course of gap junction formation? (3) Do cardiomyocytes also couple to cells from the nonmyocyte compartment? (4) Is there full functional integration also with respect to the mechanical cell-to-cell junctions? Discussion of these topics will not only be relevant with respect to host-donor cell interaction in cellular transplantation but also to explain the observation that functional recoupling of myocardial tissue is observed between host and donor hearts in about 10% of whole-heart transplantation.6

An essential difference seems to exist between recoupling of adult and neonatal/embryonic cardiomyocytes. If neonatal cardiomyocytes are seeded individually on cultured substrates, formation of gap junction occurs rapidly, the first junctions are observed within tens of minutes.7 The types of connexins found in culture correspond to the connexins synthesized in vivo.8,9 Cell-to-cell recoupling between adult cardiomyocytes is more complex. After enzymatic digestion, adult cardiomyocytes keep their normal structure and function for a few hours. Subsequently, dedifferentiation and redifferentiation change the cell phenotype, a process that has been well defined in rats.10 First signs of dedifferentiation are already observed within the first 24 hours (loss of functionally connected t-tubular membrane11). During redifferentiation, cell-to-cell junctions containing phosphorylated Cx43 are first observed after about 4 to 6 days,12,13 ie, considerably later than in cultures of neonatal cells. Although the analysis of time course of donor cell integration into the host tissue was not a major goal of the study by Rubart et al, the authors report full cell integration after 8 to 37 days. This time might be of future therapeutic relevance, if corresponding delays will occur in larger mammals.

Interestingly, the introduction of gap junctions between adjacent cell membranes is only observed after the formation of mechanical junctions (visualized by N-cadherin immunofluorescence12). This points to an at least partial association between the formation of mechanical and electrical cell-to-cell junctions. Similarly, close associations between regulation of mechanical and gap junctions can also be observed in genetic defects of mechanical junctions14 and very early during acute application of mechanical pulsatile strain.15 Since mechanical junctions ensure the function of a contiguous mechanical syncytium (by linking the contractile apparatus of adjacent cells), it would be interesting to gather

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Department of Physiology, University of Bern, Switzerland.

Correspondence to André G. Kléber, MD, Department of Physiology, University of Bern, Bühlplatz 5, CH-3012 Bern, Switzerland. E-mail kleber@pyl.unibe.ch

(Circ Res. 2003;92:1176-1178.)

© 2003 American Heart Association, Inc.

Circulation Research is available at http://www.circresaha.org

DOI: 10.1161/01.RES.0000078363.00256.D7
complementary information related to the mechanical coupling between host and donor cells. Full functional integration of embryonic donor cells into adult ventricular myocardium also implicates that the (not fully mature) fusiform donor cells change into a rod-shaped architecture and form adult-type gap junctions with their host cells. In adult myocardium, gap junctions are concentrated at the cell poles, the majority of gap junctions being localized in the terminal 20% of cell length.16 Neonatal tissue shows a different type of connexin distribution. Here, gap junctions are small and distributed at regular intervals around the cell perimeter.17 A pattern similar to neonatal tissue can be observed in disease, eg, in the remodeled zone surrounding myocardial infarction. In rat, the transition from neonatal to adult type was observed to progress during 90 postnatal days.18 In the study by Rubart et al,19 integration of embryonic donor myocytes into host tissue appears to have changed the embryonic shape of cardiomyocytes to the adult type within a very short time. With respect to the adaptation of gap junction distribution pattern, the situation is less evident. In Figure 4 of Rubart et al,19 at least one cell shows a concentration of junctions at the cell poles, whereas the other junctions show a more regularly distributed dot-like appearance. The difference in distribution pattern of gap junctions is likely to have a significant, albeit moderate effect on electrical function.17

As stated in Rubart et al,19 cellular transplantation in heart has been carried out with a variety of cell types, including skeletal myoblasts, adult stem cells, and endothelial cell precursors. It remains to be shown to what degree and quality these cell types establish cell-to-cell coupling to host cardiomyocytes. Hyde et al20 documented the existence of gap junctions between myocytes and fibroblasts in culture using electrophysiological and morphological methods already in 1969. While this is a general finding in cell culture, no consistent coupling by gap junctions was found in situ between cells of the sinoatrial node and apposed fibroblasts.21 Fibroblasts in culture show a slow rising membrane potential transmitted passively by the action potentials of surrounding cells, and produce local delays in propagation, because of the electrical current sink caused by the interspersed electrically coupled but inexcitable cells.23 This points to the fact that host cells must exhibit two qualities to fully integrate into host tissue, a degree of electrical coupling similar to normal myocytes and a phenotype that can support normal electrical efficiency.22

In the work of Rubart et al,19 the evidence that embryonic donor myocytes are electrically coupled to host myocytes is compelling but indirect. Important, at least qualitatively and within the limits of resolution of the method, is the observation that the Ca2+ transients in ventricular donor cells, elicited by normal electrical propagation, show a shape identical to Ca2+ transients in the host cells with no major detectable time delay. Theoretically, two alternative mechanisms may be suggested to explain the induction of Ca2+ transients without the transmission of the electrical impulse through gap junctions and subsequent electromechanical coupling. First, it may be argued that stretch of the host cells with subsequent release might lead to Ca2+ release and a subsequent Ca2+ transient in the donor cell. This possibility is unlikely to explain the observations of Rubart et al, because the transients of host and donor cells occur in phase. Also, this mechanism has recently been excluded in a cell culture model where HeLa cells were interspersed between two segments of culturated myocytes. These nonmyocyte “bridges” only transmitted the electrical impulse if they were transfected to produce Cx43.25 A further potential mechanism explaining cellular excitation in absence of connexins is the so-called field effect transmission.26 This transmission can be simulated in a computer with certain restricted assumptions about the electrical resistances of the intercellular clefts and the distribution of Na+ channels (clustering of channels at the intercalated disks).27 Thus far, it has been proposed that this mechanism could have a supportive role in slow conduction, as found in certain pathological states; however, it has not been given a role in normal cell-to-cell transmission of excitation.

In summary, the work of Rubart et al demonstrates elegantly and using sophisticated methodology that myogenic donor cells can show an almost perfect morphological and functional integration into genetically homologous host tissue in vivo. As stated by these authors, several obstacles need to be overcome on the way toward cellular replacement therapy. In the case of embryonic myocyte injection, the percentage of injected cells that eventually embed in host myocardium is still low, and further research is necessary to improve the efficiency.

Acknowledgments

This work was supported by the Swiss National Science Foundation and the Swiss Heart Foundation.

References


**Key Words:** cell transplantation, intercellular junctions, connexins, Ca\textsuperscript{2+} transients
Cell-to-Cell Coupling Between Host and Donor Cells in the In Situ Myocardium
André G. Kléber

Circ Res. 2003;92:1176-1178
doi: 10.1161/01.RES.0000078363.00256.D7

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/92/11/1176